U.S. DEPARTMENT OF COMMERCE
National Technical Information Service

PB-287 345

Autotrophic Denitrification Using Sulfur Electron Donors

Cornell Univ, Ithaca, NY

Prepared for

Municipal Environmental Research Lab, Cincinnati, OH

Jul 78

United States
Environmental Protection
Agency

Municipal Environmental Research
Laboratory
Cincinnati OH 45268

EPA-600/2-78-113
July 1978

Research and Development

PB 287345

EPA

Autotrophic Denitrification Using Sulfur Electron Donors

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U. S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

TECHNICAL REPORT DATA (Please read Instructions on the reverse before completing)		
1. REPORT NO. EPA-600/2-78-113	2.	3. RECIPIENT'S ACCESSION NO. PB287345
4. TITLE AND SUBTITLE AUTOTROPHIC DENITRIFICATION USING SULFUR ELECTRON DONORS		5. REPORT DATE July 1978 (Issuing Date)
		6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Alonzo Wm. Lawrence, James J. Bisogni, Jr., Bill Batchelor and Charles T. Driscoll, Jr.		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Dept. of Civil & Environmental Engineering Hollister Hall Cornell University Ithaca, New York 14853		10. PROGRAM ELEMENT NO. 1BC611, SOS #3, Task C/03
		11. CONTRACT/GRANT NO. Grant #803505
12. SPONSORING AGENCY NAME AND ADDRESS Municipal Environmental Research Laboratory--Cin.,OH Office of Research and Development U.S. Environmental Protection Agency Cincinnati, Ohio 45268		13. TYPE OF REPORT AND PERIOD COVERED 4/74 - 4/78 Final
		14. SPONSORING AGENCY CODE EPA/600/14
15. SUPPLEMENTARY NOTES Project Officer: E. F. Barth, Cincinnati, Ohio. Telephone: (513)684-7641		

16. ABSTRACT

This research project investigated the feasibility of autotrophic denitrification as a nitrate removal process for municipal wastewater. The overall objective of this project was to evaluate the microbial kinetics, and to assess the process performance of autotrophic microbially mediated denitrification using sulfur electron donors.

This study was divided into three experimental phases. Each phase utilized a different sulfur compound or flow configuration. Included in these phases were: continuous flow slurry-type with elemental sulfur as the electron source; semi-continuous flow, complete-mix reactors with thiosulfate or sulfide as the electron source; and packed bed columnar reactors with elemental sulfur as the electron source.

Based on theoretical and experimental considerations, kinetic models and stoichiometric relationships were developed for the autotrophic denitrification process.

The results of this study indicate that autotrophic denitrification with various sulfur species, particularly elemental sulfur, is a feasible scheme for removal of nitrate from wastewater effluents.

17. KEY WORDS AND DOCUMENT ANALYSIS		
a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Wastewater* Nitrogen Cycle* Sulfate Reducing Bacteria*	Various sulfur species Methanol replacement* Suspended growth reactor Packed column reactor Stack gas sulfur	13B
18. DISTRIBUTION STATEMENT Release to Public	19. SECURITY CLASS (This Report) Unclassified	21. NO. OF PAGES 129
	20. SECURITY CLASS (This page) Unclassified	22. PRICE PC A07

EPA Form 2220-1 (9-73)

* U.S. GOVERNMENT PRINTING OFFICE: 1978— 757-140/1420

RESEARCH REPORTING SERIES

Research reports of the Office of Research and Development, U.S. Environmental Protection Agency, have been grouped into nine series. These nine broad categories were established to facilitate further development and application of environmental technology. Elimination of traditional grouping was consciously planned to foster technology transfer and a maximum interface in related fields. The nine series are:

1. Environmental Health Effects Research
2. Environmental Protection Technology
3. Ecological Research
4. Environmental Monitoring
5. Socioeconomic Environmental Studies
6. Scientific and Technical Assessment Reports (STAR)
7. Interagency Energy-Environment Research and Development
8. "Special" Reports
9. Miscellaneous Reports

This report has been assigned to the ENVIRONMENTAL PROTECTION TECHNOLOGY series. This series describes research performed to develop and demonstrate instrumentation, equipment, and methodology to repair or prevent environmental degradation from point and non-point sources of pollution. This work provides the new or improved technology required for the control and treatment of pollution sources to meet environmental quality standards.

This document is available to the public through the National Technical Information Service, Springfield, Virginia 22161.

EPA-600/2-78-113
July 1978

AUTOTROPHIC DENITRIFICATION USING SULFUR ELECTRON DONORS

by

Alonzo Wm. Lawrence,
James J. Bisogni, Jr., Bill Batchelor and Charles T. Driscoll, Jr.
Cornell University
Ithaca, New York 14853

Grant No. 803505

Project Officer

E. F. Barth
Wastewater Research Division
Municipal Environmental Research Laboratory
Cincinnati, Ohio 45268

MUNICIPAL ENVIRONMENTAL RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268

DISCLAIMER

This report has been reviewed by the Mun'cipal Environmental Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the U.S. Enviror ental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

FOREWORD

The Environmental Protection Agency was created because of increasing public and government concern about the dangers of pollution to the health and welfare of the American people. The complexity of that environment and the interplay between its components require a concentrated and integrated attack on the problem.

Research and development is that necessary first step in problem solution and it involves defining the problem, measuring its impact, and searching for solutions. The Municipal Environmental Research Laboratory develops new and improved technology and systems for the prevention, treatment, and management of wastewater and solid and hazardous waste pollutant discharges from municipal and community sources, for the preservation and treatment of public drinking water supplies, and to minimize the adverse economic, solcial, health, and aesthetic effects of pollution. This publication is one of the products of that research; a most vital communications link between the researcher and the user community.

This report summarizes the results of a feasibility study to determine if various species of sulfur could serve as substrate for biological denitrification of municipal wastewater effluent.

Francis T. Mayo, Director
Municipal Environmental Research
Laboratory

ABSTRACT

This research project investigated the feasibility of autotrophic denitrification as a nitrate removal process for municipal wastewater. The overall objective of this project was to evaluate the microbial kinetics, and to assess the process performance of autotrophic microbially mediated denitrification using sulfur electron donors.

This study was divided into three experimental phases. Each phase utilized a different sulfur compound or flow configuration. Included in these phases were: continuous flow slurry-type with elemental sulfur as the electron source; semi-continuous flow, complete-mix reactors with thiosulfate or sulfide as the electron source; and packed bed columnar reactors with elemental sulfur as the electron source.

Based on theoretical and experimental considerations, kinetic models and stoichiometric relationships were developed for the autotrophic denitrification process.

The results of this study indicate that autotrophic denitrification with various sulfur species, particularly elemental sulfur, is a feasible scheme for removal of nitrate from wastewater effluents.

This report was submitted in fulfillment of Grant No. 803505 by Cornell University, Ithaca, N.Y. under partial sponsorship of the U.S. Environmental Protection Agency.

CONTENTS

Foreword iii
Abstract iv
Figures viii
Tables x
Symbols xii

1. Introduction 1
2. Conclusions 3
3. Recommendations 5
4. Background 6
5. Theoretical Considerations 8
   Development of kinetic model 8
   Stoichiometry of autotrophic denitrification 15
      General concepts of microbial stoichiometry 15
      Thermodynamic based predictions of microbial stoichiometry 17
      Theoretical prediction of autotrophic denitrification stoichiometry 18
6. Analytical Techniques 22
7. Experimental Studies 26
   Phase I - Slurry reactors using elemental sulfur 26
      Cultural characterization experiments 26
         Experimental plan 26
         Experimental techniques 27
         Experimental results 29
            Variable sulfur to nitrate-nitrogen feed ratio 29
            Variable ammonia-nitrogen to nitrate-nitrogen feed ratio 29
            Variable temperature study 31
            Batch stoichiometry 33
      Continuous culture experiments 34
         Experimental plan 34
         Experimental techniques 34
            Reactor operations 34
            Steady state techniques 38
            Transient rate study techniques 39
            Solids separation study techniques 40
         Experimental results 41
            Variable solids retention time 41
            Variable sulfur to biomass ratio 41
            Effect of temperature 45
            Settling and thickening characteristics . . . 45

Discussion of Phase I experimental results. 54
Observed yield coefficients. 54
Balanced stoichiometric equation 54
Sulfur balance 56
Composition of reactor gas 56
Kinetics 56
Temperature effects. 58
Settling and thickening 59
Phase II - Thiosulfate and sulfide experiments. 60
Experiment #1 - effect of growth rate - high C_F 60
Experimental plan and techniques 60
Experimental results 60
Experiment #2 - effect of growth rate - intermediate C_F. 64
Experimental plan and techniques 64
Experimental results 64
Experiment #3 - determination of consumptive ratio . . . 64
Experimental plan and techniques 64
Experimental results 64
Experiment #4 - sulfide experiments. 64
Experimental plan and techniques 64
Experimental results 64
Discussion of Phase II experimental results 66
Thiosulfate and sulfide as electron donors 66
Observed yield and consumptive ratio 66
Phase III - Packed bed reactor experiments. 67
Experiment #1 - dolomitic limestone reactors - sulfide feed 67
Experimental plan and techniques 67
Experimental results 70
Experiment #2 - elemental sulfur packed bed reactors . . 70
Experimental plan and techniques 70
Experimental results 70
Experiment #3 - sulfur-dolomite packed bed studies . . . 70
Experimental plan and techniques 70
Experimental results 74
Experiment #4 - packed bed studies with domestic wastewater effluent 75
Experimental plan and techniques 75
Experimental results 75
Discussion of Phase III experimental results 77
Elemental sulfur packed bed performance. 7
Alkalinity supplementation with dolomite 8
Denitrification of domestic secondary effluent . . . 8:
8. Engineering Significance 83
Evaluation of sulfur-substrates. 83
Technical feasibility. 85
Cost of electron donor 86
Cost of supplemental alkalinity 87
Sludge disposal. 87
Environmental impact 88

Summary 88
References 89
Appendices 95
A. Elemental sulfur analysis, ATP analysis 95
B. Transient rate test data, results of zone settling test, results of flocculent settling test. 98

FIGURES

Number		Page
1	Schematic of sulfur-biofilm system with zeroth-order nitrate rate limitation.	9
2	Hyperbolic tangent and Monod functions versus ζ(S/X).	11
3	Schematic of slurry reactor system.	13
4	Proposed electron flow for autotrophic denitrification under anaerobic conditions with water the electron donor for synthesis.	19
5	Schematic of cadmium reduction column used for nitrate analysis	24
6	Schematic of semicontinuous culture reactor system.	28
7	Rate of gas production versus sulfur to nitrate-nitrogen feed ratio.	30
8	Rate of gas production versus ammonia-nitrogen to nitrate-nitrogen feed ratio.	32
9	Schematic of continuous culture reactor system.	36
10	Observed biomass yield versus solids retention time	43
11	ATP content of biomass versus solids retention time	44
12	Maximum attainable unit rate of denitrification versus sulfur to biomass ratio	47
13	Natural logarithm of maximum attainable unit rate of denitrification versus inverse of absolute temperature.	49
14	Solids flux versus solids concentration for various values of the sulfur to biomass ratio.	50
15	Suspended solids concentration on depth versus time graph with lines of isoconcentration	51

Number		Page
16	Effluent suspended solids concentration versus overflow rate for various values of the sulfur to biomass ratio.	52
17	Semicontinuous flow sulfide/thiosulfate reactor	62
18	Response of autotrophic denitrifying system to rapid changes in feed ratio.	65
19	Schematic of continuous flow sulfide feed packed bed reactor system	69
20	Schematic of continuous flow sulfur packed bed reactor system .	71
21	Effluent nitrate concentration as a function of hydraulic retention time for different particle size reactor media . .	73
22	Effluent quality as a function of hydraulic retention time. . .	78
23	Minimum hydraulic retention time for complete denitrification as a function of estimated sulfur surface area.	79
24	Feed alkalinity versus apparent alkalinity consumption for sulfur packed bed reactors supplemented with dolomite. . . .	81

TABLES

Number		Page
1	Summary of Steady-State Material Balances for Autotrophic Denitrification in a Slurry Reactor System.	14
2	Summary of Analytical Techniques	23
3	Summary of Experimental Plan	27
4	Composition of Media for Semicontinuous Culture Experiments. .	29
5	Effect of Sulfur to Nitrate Feed Ratio on Gas Production Rate.	31
6	Effect of Ammonia to Nitrate Feed Ratio on Gas Production Rate.	31
7	Effect of Temperature on Gas Production Rate	33
8	Batch Stoichiometric Coefficients.	34
9	Summary of Experimental Plan for Continuous Culture Experiments	35
10	Composition of Continuous Culture Feed Solution.	39
11	Summary of Continuous Culture Results at Various θ_c	42
12	Summary of Continuous Culture Results at Various S/X	46
13	Summary of Continuous Culture Results at Various Temperatures.	48
14	Regression Equations for Results of Solids Separation Tests. .	53
15	Stoichiometric Coefficients for Batch and Continuous Cultures.	55
16	Experimental Program - Phase II (Steady-State)	60
17	Phase II - Feed Solution Nutrients	61

18 Summary of Steady-State Experimental Results - Phase II. . . . 63

19 Phase III Feed Characteristics (Expts. #1, #2, #3) 68

20 Experiment #1 - Phase III Steady-State Experimental Results. . 70

21 Phase III Experiment #2 Reactor Characteristics. 72

22 Phase III Experiment #3 Reactor Characteristics. 74

23 Experiment #3 - Phase III Results - Sulfur/Dolomite Reactor. . 74

24 Experiment #3 - Phase III Results-Sulfur Reactor 75

25 Experiment #4 - Phase III Sulfur/Dolomite Packed Bed Reactor Performance with Secondary Effluent Feed (θ = 27.8 Hrs) . . 76

26 Experiment #4 - Phase III Sulfur Packed Bed Reactor Performance with Secondary Effluent Feed (θ = 21.1 Hrs) 76

27 A Comparison of Measured and Theoretical Sulfate Production to Nitrate Reduction Ratio. 82

28 Comparison of Costs of Sulfur Substrates and Methanol. 84

LIST OF SYMBOLS

A,B,C,D	= symbols for generalized chemical compounds
A_c	= surface area of clarifier-thickener, [m^2]
ATP	= adenosine triphosphate, primary energy storage compound in cells
c	= ratio of elemental sulfur to nitrate-nitrogen feed rate, [mg/mg].
C_F	= ratio of electron equivalents of electron donor (sulfur compound) in feed to electron equivalents of electron acceptor in feed
C_R	= ratio of electron equivalents of thiosulfate or sulfide consumed to electron equivalents of nitrate reduced
C_1, C_2	= concentrations of elemental sulfur and nitrate-nitrogen within the biofilm, respectively; superscripted with o, ', or δ to denote evaluation at Z=0, Z=Z', or Z=δ, respectively, [mg/ℓ]
CEN	= equivalent nitrate-nitrogen concentration, [mg/ℓ]
DO	= dissolved oxygen concentration [mg/ℓ]
E_a	= Arrhenius activation energy, [kcal/mole]
f_e^a, f_s	= fraction of electron equivalents in observed reaction allocated to energy and synthesis subreactions, respectively
ΔG	= change in Gibbs free energy, [kcal/mole]
ΔG^o, ΔG_e^o, ΔG_p^o, ΔG_{so}^o, ΔG_{sn}^o	= change in Gibbs free energy measured under standard conditions in general, for oxidation of one electron equivalent by energy reaction, for conversion of one electron equivalent of carbon source to pyruvate, for oxidation of one electron equivalent of sulfur by oxygen, and for oxidation of one electron equivalent of sulfur by nitrate, respectively
ΔG_c^o	= ATP-energy required to convert one electron equivalent of pyruvate to cell material, [kcal/electron equivalent]
k	= efficiency factor for microbial energy conversion
K	= zeroth-order rate constant for removal of CEN during transient rate tests, [mg/ℓ-d]
K_n	= saturation coefficient in function expressing dependence of unit rate of denitrification on nitrate-nitrogen concentration, [mg/ℓ]
m	= coefficient indicating whether energy is released (-1) or (+1) by reaction which converts carbon source to pyruvate

M	= slope of solids flux versus total solids concentration curve, [m/d]
N, N^o	= concentration of nitrate-nitrogen in reactor and in influent, respectively [mg/ℓ]
NI	= concentration of nitrite-nitrogen, [mg/ℓ]
NFN	= function expressing dependence of unit rate of denitrification on nitrate-nitrogen concentration
Q	= volumetric flow rate for influent, [ℓ/d]
Q/A_c	= surface overflow rate for clarifier-thickener, may be superscripted with max to denote the maximum value attainable under specified operating conditions, [m^3/m^2-d]
r	= ratio of recycle flow rate to influent flow rate
R	= universal gas constant, [kcal/mole-^oK]
R_n, R_s, R_x	= volumetric rates of removal of nitrate-nitrogen and elemental sulfur, and production of biomass, respectively, [mg/ℓ-d]
S, S^o, S^r, S^{eff}	= concentration of elemental sulfur in reactor, influent, recycle, and effluent, respectively, [mg/ℓ]
S/X	= ratio of concentration of elemental sulfur to concentration of biomass, [mg/mg]
T	= temperature, [^oK]
U	= unit rate of denitrification, equal to rate of nitrate-nitrogen removal divided by biomass concentration, [mg/mg-d]
U_{max}	= maximum unit rate of denitrification at a specified temperature, [mg/mg-d]
$U_{m,a}$	= maximum attainable unit rate of denitrification at a specified value of S/X, and temperature, [mg/mg-d]
U_o	= coefficient in Arrhenius equation for temperature dependence of unit rate of denitrification, [mg/mg-d]
V	= volume of reactor, [ℓ]
w	= volumetric flow rate in wastage line, [ℓ/d]
X, X^r, X^{eff}	= biomass concentration in reactor, recycle, and effluent, respectively, [mg/ℓ]
X_t^a, X_t^r	= total solids concentration in reactor and recycle, respectively, [mg/ℓ]
Y_{obs}	= observed biomass yield, equal to rate of biomass production divided by rate of nitrate-nitrogen removal, [mg/mg]
Z	= distance into biofilm from sulfur surface, [m]
ZSV	= zone settling velocity measured in batch settling tests, [m/d]
δ	= biofilm thickness, [m]
ΔAlk	= decrease in alkalinity concentration, [mg/ℓ]
ΔSO_4-S	= increase in sulfate-sulfur concentration, [mg/ℓ]
θ	= hydraulic retention time, equal to reactor volume divided by influent flow rate, [d]
θ_c	= solids retention time, equal to total amount of biomass divided by rate at which biomass is removed from system [d]

$\theta_c^{m,a}$ = minimum attainable solids retention time, corresponds to maximum attainable unit rate of denitrification, [d]

υ = stoichiometric coefficient equal to rate of sulfur removal divided by rate of nitrate-nitrogen removal, [mg/mg]

ζ = kinetic coefficient which depends on sulfur particle geometry, biomass density, and intra-film kinetic and transport coefficients, [mg/mg].

SECTION 1

INTRODUCTION

Concern with nutrient enrichment of natural waters and safety of drinking water supplies has stimulated recent research and development of biological denitrification processes. At the present time (1978) the most highly developed denitrification process employs heterotrophic organisms in the final stage of a multiple stage reactor system. Because the influent to the denitrification step contains essentially refractory organics, an exogenous supply of organic compounds (typically, methanol) must be added to supply energy for the microbial denitrification process. However, recent political-economic events have resulted in rapid increases in the cost of crude oil and concomitant decreased availability of methanol and other organic chemicals. Thus, it becomes attractive to consider alternative methods of denitrification.

An alternative to heterotrophic biological nitrate removal could employ an enrichment culture of Thiobacillus denitrificans in an autotrophic denitrification process. This organism does not require organic compounds and can reduce nitrate to nitrogen gas while oxidizing a wide variety of sulfur compounds ($S^{=}$, S^{o}, $S_2O_3^{=}$, $S_4O_6^{=}$, $SO_3^{=}$) to sulfate. T. denitrificans is autotrophic since it uses inorganic carbon as its source of carbon for cell synthesis.

Autotrophic denitrification processes can be categorized according to sulfur source and reactor configuration. Elemental sulfur appears to be the sulfur compound most likely to be feasible in a full scale process due to its low cost, ease of storage and handling, and lack of toxicity. Other forms of sulfur such as thiosulfate or sulfide might also be practical, especially if industrial wastes containing these compounds were available. Such soluble sulfur compounds could also be expected to sustain higher rates of denitrification than elemental sulfur. It is anticipated that slurries, packed beds, and expanded beds could all be used as reactor configurations for autotrophic denitrification.

The primary advantage of an autotrophic denitrification process over heterotrophic processes is the expected cost of supplying electron donors. Sulfur is now relatively inexpensive (1978) and widespread adoption of sulfur oxide removal technology for combustion stack gases would mitigate against any future price increase. It may be possible to link sulfur oxide removal and autotrophic denitrification either directly by having the sulfur removal process supply the wastewater treatment plant, or indirectly by the effect on the sulfur market of increased supplies from stack gas recovery facilities.

The amount of sulfur which could be made available from sulfur oxide control is significant. In 1968, emissions of sulfur from U.S. power plants was estimated as 12.2 million tons, while the total U.S. commercial sulfur production was 10.4 million tons.

Autotrophic denitrification processes also may have certain disadvantages. These processes would enrich the wastewater in sulfate and destroy alkalinity. Sulfate enrichment might be a problem due to deterioration of water quality caused by the elevated sulfate concentration itself, or by its stimulatory effect on microbial sulfide production.

Despite the potential attractiveness of autotrophic sulfur-oxidizing denitrification, little quantitative information was available on which to base judgments concerning the practical feasibility of the process. Thus, the overall objectives of this project were to: 1) delineate the kinetic, stoichiometric and solids separation characteristics of an autotrophic denitrification process using elemental sulfur in a slurry reactor system; 2) determine the feasibility of employing soluble sulfur species, thiosulfate and sulfide, in completely mixed semi-continuous flow denitrification systems; and, 3) investigate autotrophic denitrification using sulfur in packed bed reactor configurations.

SECTION 2

CONCLUSIONS

The results of the three experimental phases of this project indicate that autotrophic denitrification using sulfur electron donors is a feasible alternative technology for wastewater nitrate removal.

The first experimental phase involved continuous flow, slurry type reactors, with elemental sulfur as the electron source. From this phase it was concluded that:

1. Essentially complete nitrate removal (>99.5 percent) can be attained at steady state.

2. The effect of nitrate concentration on the unit rate of denitrification can be estimated by a Monod function with a saturation constant equal to 0.03 mg/l NO_3^--N.

3. The maximum attainable unit rate of nitrate removal is a linear function of the ratio of reactor sulfur concentration (S) to reactor biomass organic nitrogen concentration (X) over the range S/X = 45 - 194 mg S/mg organic-N.

4. Temperature dependence of the maximum attainable unit rate of denitrification over the range 12-30°C can be described by the Arrhenius equation with an activation energy of 13.2 kcal/mole.

5. Stoichiometry for autotrophic denitrification is relatively constant over a range of solids retention times (7.6-30 days), values of S/X (45-194 mg S/mg organic-N), and temperatures (12-30°C), and can be represented by the following equation:

$$1.0\ NO_3^- + 1.10\ S + 0.40\ CO_2 + 0.76\ H_2O + 0.080\ NH_4^+$$
$$\rightarrow 0.080\ C_5H_7O_2N + 0.50\ N_2 + 1.10\ SO_4^= + 1.28\ H^+$$

6. Solids flux is a linear function of solids concentration for sulfur-biomass slurries, with smaller fluxes at lower values of S/X.

7. The concentration of effluent suspended solids is a linear function of overflow rate, with smaller concentrations at lower values of S/X.

8. Economic feasibility of autotrophic denitrification will depend to a great extent on the relative prices of elemental sulfur and methanol.

The second experimental phase employed semi-continuous flow reactors, with thiosulfate or sulfide as the electron source. From this phase it was concluded that:

1. Reliable autotrophic denitrification can be obtained using thiosulfate or sulfide as electron donors.

2. The consumptive ratio for these systems appears to be close to 1.35, the thermodynamically predicted value.

3. Thiosulfate systems could be maintained with feed ratios as low as 0.45 with no apparent inhibition of denitrification. In addition these systems could be changed between thiosulfate and nitrate limiting growth conditions without affecting the stability of the system.

In the final experimental phase, packed bed columnar autotrophic denitrification was studied. From this phase it was concluded that:

1. Autotrophic denitrification is possible in packed bed reactors using elemental sulfur as an electron source.

2. In reactors packed with elemental sulfur there existed a strong correlation between sulfur particle size and minimum hydraulic retention time necessary for complete denitrification.

3. Alkalinity consumption is an inherent characteristic of the autotrophic denitrification process. Dolomite can be mixed with elemental sulfur in packing media of the denitrification reactors to provide alkalinity.

4. Autotrophic denitrification can proceed in the presence of organics (and hence, heterotrophic denitrification) in packed bed reactors.

SECTION 3

RECOMMENDATIONS

Investigation of autotrophic denitrification as a nitrate removal process should continue. The study reported herein showed favorable results and it appears that autotrophic denitrification is a feasible process.

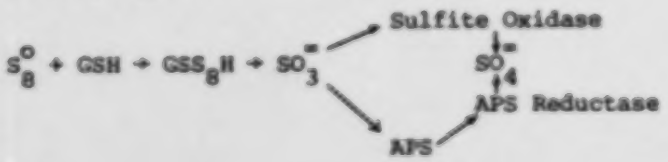
Primarily, what remains to be investigated is the performance of the scheme on a pilot scale basis. It appears that elemental sulfur is the most practical electron donor. Hence, pilot-scale investigations should include a study of the effect of sulfur particle size (in both slurry and packed bed configurations) on process performance. The long term effect of organic matter (and hence heterotrophic denitrification) and suspended solids in feed streams such as nitrified effluent from municipal plants must also be investigated.

SECTION 4

BACKGROUND

Thiobacillus denitrificans, the microorganism responsible for autotrophic denitrification, is a gram negative motile rod (0.5 x 1.0 μm) which does not form spores (1). A wide range of reduced sulfur compounds ($S^{=}$, S^{o}, $S_2O_3^{=}$, $S_4O_6^{=}$, $SO_3^{=}$) can be oxidized by this organism to obtain energy (1). Sulfate is the normal end-product, as intermediates do not accumulate under optimal growth conditions (2). Oxygen, nitrate, nitrite, nitric oxide, and nitrous oxide can serve as terminal electron acceptors for sulfur oxidation. The nitrogen compounds will be reduced completely to nitrogen gas, except when growth is under conditions of extreme stress (3), such as in the presence of toxic substances (1). Since this microorganism prefers to use oxygen rather than nitrate as a terminal electron acceptor, denitrification should only be expected under anaerobic conditions. The pH range for growth of Thiobacillus denitrificans is between pH 6 and 8, and the optimum has been reported both on the acid and the alkaline side (1,4). Certain strains are tolerant of high concentrations of metals which would normally be toxic (5). Nitrite (1) and pyruvate (6) are inhibitory to growth. Certain keto acids which are inhibitory to other species of thiobacilli are probably also toxic to T. denitrificans (7). Soluble organic compounds have been found to be excreted by thiobacilli during growth (6). As much as 20 percent of the inorganic carbon fixed by T. denitrificans can appear in the culture media (8). Although classified as obligately autotrophic (2), there have been some reports of T. denitrificans growing on organic compounds (6,9).

Several reviews have been published dealing with the biochemistry of sulfur oxidation by thiobacilli (10,12). The proposed pathway for elemental sulfur oxidation involves reduced glutathione (GSH), which combines with elemental sulfur to form a polysulfide that is oxidized to sulfite. In aerobic thiobacilli, the enzyme responsible for this step has been isolated and found to require molecular oxygen. Therefore, most of the energy from sulfur oxidation comes from the oxidation of sulfite (12). Two methods of sulfite oxidation have been observed in T. denitrificans. A substrate level phosphorylation is involved in one of the steps with adenosine phosphosulfate (APS) as an intermediate (13,14). The other pathway is cytochrome-linked and involves only oxidative phosphorylation (10).



(1)

The physical form of sulfur has a great influence on the rate of its uptake. Smaller, wettable forms can be expected to be much more available to the microorganism. Colloidal sulfur is oxidized almost as fast as soluble sulfur compounds (12).

The exact method by which sulfur is transported into the cell is not known, but two hypotheses have been made (12). One scheme postulates a water-soluble extracellular carrier enzyme which transports the water-insoluble sulfur into the cell. Since well washed cells oxidize sulfur at linear rates without a lag period (15), this hypothesis is probably incorrect. The second mechanism involves a reaction between sulfur and a cellular component at the cell wall-sulfur interface. This explanation blends well with the postulated sulfur oxidation pathway, if a membrane-bound thiol can be substituted for GSH (12). Examination of the cell surface with an electron microscope indicates the existence of an intermediate of sulfur oxidation which apparently contains a thiol group (16).

Intermediates of nitrate reduction by T. denitrificans have been reported as nitrite, nitric oxide, and nitrous oxide (17).

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2^o \qquad (2)$$

An electron transport system (ETS) with cytochromes is involved in nitrate reduction (18-21). Inhibition of nitrate reduction by oxygen indicates the existence of two ETS or a branched system (22,23). The reduction of nitrite to nitrogen gas, however, does not seem to be linked to cytochromes (17).

The Calvin cycle for carbon dioxide reduction used by photosynthetic cells is the pathway found in thiobacilli (24). Although some tricarboxylic acid cycle enzymes are present (25) they are used for biosynthetic purposes (26).

At the time (1974) work was initiated on this project, there was only one report in the literature on the application of this microbial phenomenon to wastewater treatment. Gram (27) had described a laboratory feasibility study in which a simulated agricultural drainage water was successfully denitrified using microbially active anaerobic columns packed with elemental sulfur.

SECTION 5

THEORETICAL CONSIDERATIONS

DEVELOPMENT OF KINETIC MODEL

A mathematical model was developed to describe microbial growth on solid, water-insoluble substrates at high biomass densities in order to describe the kinetic behavior of autotrophic denitrification processes using elemental sulfur. This model was based on a material balance about a differential section of biofilm attached to a sulfur particle. Figure 1 is a schematic representation of such a system, where C_1 and C_2 represent the concentrations of sulfur and nitrate within the biofilm. Distance into the biofilm from the sulfur surface is represented by Z, and the total biofilm thickness is represented by δ. Sulfur is solubilized in the biofilm at $Z = 0$ at a concentration of C_1^o, and is biologically oxidized while being transported through the film.[1] Sulfur does not leave the biofilm and enter the bulk liquid at $Z = \delta$, since it is insoluble in water. Nitrate enters the biofilm at $Z = \delta$ at a concentration of C_2^δ, and is similarly removed while being transported through the film. At the point $Z = Z'$, the intra-film nitrate concentration goes to zero, so no reaction occurs in the region $0 < Z < Z'$, i.e., between the sulfur surface and the point where the nitrate concentration goes to zero.

The concurrent transport and reaction of sulfur and nitrate within the biofilm was modelled by assuming that the transport of both species occurs in a manner analogous to molecular diffusion. Rates of sulfur and nitrate removal were related by a constant stoichiometry, and were assumed to be proportional to the intra-film sulfur concentration. These rates were considered independent of the intra-film nitrate concentration as long as intra-film nitrate concentration remained non-zero. However, the observed rate is affected by the concentration of nitrate in the bulk liquid through limitations on intra-film transport of nitrate. These limitations occur when the bulk liquid nitrate concentration is low and the biofilm is thick. Such a case is depicted in Figure 1 which shows nitrate penetrating through only a portion of the biofilm. As the bulk liquid nitrate concentration increases, a larger portion of the biofilm becomes active. At some bulk liquid nitrate concentration, nitrate will penetrate throughout the biofilm. Further increases in bulk liquid nitrate concentration will not increase the rate of denitrification because the entire biofilm is active. Other assumptions used in the development of this model were that biomass is evenly distributed over the sulfur surface; and that the biomass density, transport coefficients, and ratio of mass to surface area for the sulfur particles are constant.

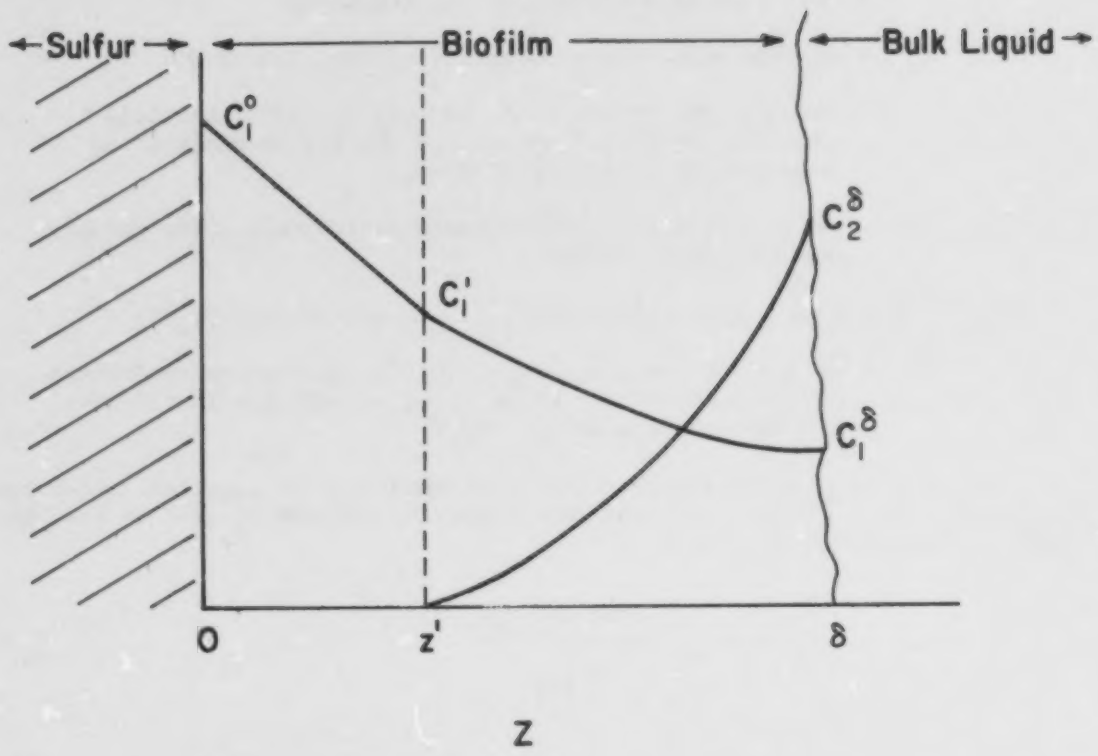


Figure 1. Schematic of sulfur-biofilm system with zeroth-order nitrate rate limitation.

The equation resulting from a differential material balance on a section of the biofilm was combined with a material balance over the entire biofilm to produce a kinetic equation for the unit rate of denitrification.

$$U = U_{max} \left[\frac{\tanh \left(\frac{1}{\zeta \, S/X} \right)}{\left(\frac{1}{\zeta \, S/X} \right)} \right] \cdot \left[\frac{N}{K_n + N} \right] \qquad (3)$$

U = unit rate of denitrification, [mg/mg·d];

U_{max} = maximum unit rate of denitrification, [mg/mg·d];

ζ = kinetic coefficient which depends on sulfur particle geometry, biomass density, and intra-film kinetic and transport coefficients, [mg/mg];

S/X = ratio of reactor sulfur concentration to reactor biomass concentration, [mg/mg];

N = bulk liquid nitrate-nitrogen concentration [mg/ℓ];

K_n = saturation coefficient in function expressing dependence of unit rate of denitrification on bulk liquid nitrate-nitrogen concentration, [mg/ℓ].

It is convenient to separate the right-hand side of Equation 3 into two functions. One of these functions represents the dependence of U on S/X; the other the dependence of U on N.

$$U_{m,a} = U_{max} \, \frac{\tanh \left(\frac{1}{\zeta \, S/X} \right)}{\left(\frac{1}{\zeta \, S/X} \right)} \qquad (4)$$

$$NFN = \frac{N}{K_n + N} \qquad (5)$$

$U_{m,a}$ = maximum attainable unit rate of denitrification, [mg/mg·d];

NFN = function expressing dependence of U on nitrate concentration (dimensionless).

The hyperbolic tangent function $\left[\frac{\tanh \left(\frac{1}{\zeta \, S/X} \right)}{\left(\frac{1}{\zeta \, S/X} \right)} \right]$ in Equation 4 is similar in form to the Monod function, which is commonly used to describe microbial kinetics. Figure 2 shows the relationship between the hyperbolic

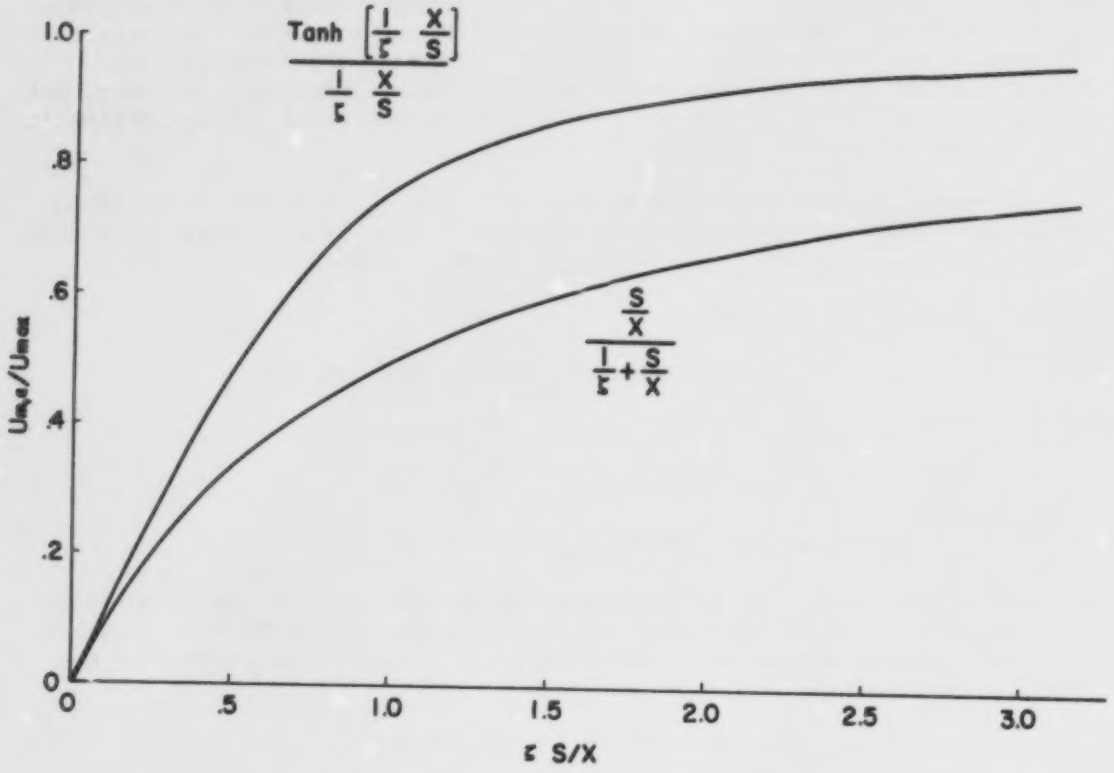


Figure 2. Hyperbolic tangent and Monod functions versus ζ(S/X).

tangent function and the Monod function with $\zeta(S/X)$ as the independent variable. Each function has a region of first-order behavior at low values of $\zeta(S/X)$, and each approaches a limiting maximum at high values. The hyperbolic tangent function, however, approaches its maximum faster and displays a larger region where the rate is proportional to $\zeta(S/X)$.

The most important aspect of this model is the conclusion that the primary kinetic variable is neither the biomass concentration nor the sulfur concentration but their ratio. For constant nitrate concentration and temperature, this ratio determines the rate of denitrification. Under relatively non-restrictive assumptions, S/X is proportional to the biofilm thickness, which is the maximum length sulfur or nitrate must travel through the film before reacting. Decreasing the biofilm thickness increases the average intra-film sulfur concentration, thereby increasing the observed rate. Sulfur surface area is the quantity which actually influences the rate, but sulfur mass can be used as a measure of surface area when the proportionality between the two remains constant.

Temperature dependence of biological rates is often expressed in an analogous manner to the temperature dependence of chemical rates, which can usually be represented by the Arrhenius equation (28,29).

$$U = U_o \exp(-E_a/RT) \tag{6}$$

E_a = Arrhenius activation energy, [kcal/mole];

R = universal gas constant, [kcal/mole-^oK];

T = absolute temperature, [^oK];

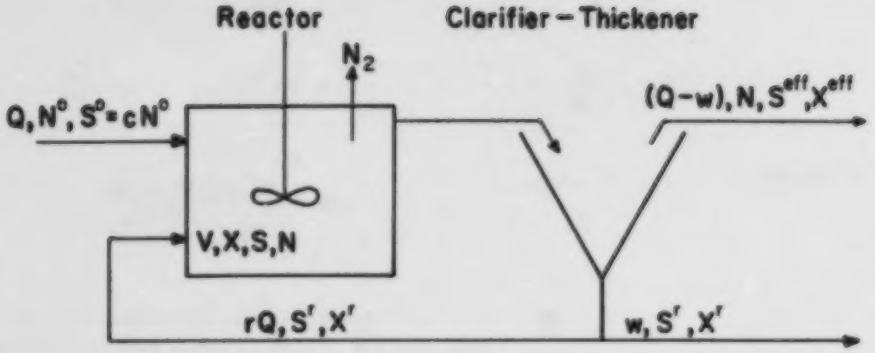
U_o = constant, [mg/mg·d].

Activation energy (E_a) is the parameter which incorporates the temperature dependence. It can be determined by fitting experimental data to a linear equation relating the natural logarithm of the rate to the inverse of the absolute temperature.

$$\ln (U) = \ln (U_o) - (E_a/R) \frac{1}{T} \tag{7}$$

Figure 3 shows a schematic of a slurry reactor system similar to the ones used in Phase I of this study. Material balances on nitrate, biomass, and sulfur can be made and the results used to apply the kinetic model to predict behavior of a slurry reactor system. Table 1 summarizes these material balance equations.

Solids separation in a slurry system is intimately linked with overall process performance. Solids must be separated from the effluent (clarification) and compacted to higher concentrations (thickening) if the process is to operate. Poor clarification decreases effluent quality by increasing suspended solids and, in the extreme, can cause system failure due to biomass



N^o, N = concentration of nitrate-nitrogen in influent and reactor respectively, (M/L^3);

S^o, S, S^r, S^{eff} = concentration of elemental sulfur in influent, reactor, recycle line, and effluent respectively, (M/L^3);

X, X^r, X^{eff} = biomass concentration in reactor, recycle line, and effluent respectively, (M/L^3);

Q, rQ, w = volumetric flow for influent, recyle and wastage flows respectively, (L^3/T).

Figure 3. Schematic of slurry reactor system.

TABLE 1. SUMMARY OF STEADY-STATE MATERIAL BALANCES FOR AUTOTROPHIC DENITRIFICATION IN A SLURRY REACTOR SYSTEM

Constituent	Material Balance	Reactor Concentration
Nitrate	$R_n = \frac{N^o - N}{\theta}$	$N = N^o - U\theta X$
Biomass	$R_x = \frac{X}{\theta_c}$	$X = Y_{obs}\ (N^o - N)\ \frac{\theta_c}{\theta}$
Sulfur	$R_s = \frac{cN^o}{\theta} - \frac{S}{\theta_c}$	$S = [N^o(c-\upsilon) + \upsilon N]\ \frac{\theta_c}{\theta}$
Sulfur to Biomass Ratio	———	$S/X = \frac{N^o(c-\upsilon) + \upsilon N}{Y_{obs}\ (N^o - N)}$

R_n, R_s, R_x = volumetric rates of removal of nitrate and sulfur, and production of biomass, respectively [mg/ℓ · d];

θ = hydraulic retention time, equal to V/Q, [d];

θ_c = solids retention time, equal to $VX/(w\ X_r + (Q-w)\ X^{eff})$, [d];

c = sulfur feed ratio, equal to ratio of sulfur feed rate to QN^o, [mg/mg];

υ = stoichiometric parameter equal to R_s/R_n, [mg/mg].

loss. Poor thickening characteristics require larger areas in the clarifier-thickener and, in the extreme, cause system failure by inhibiting clarification.

The batch flux method is a technique used to determine the thickening characteristics of a slurry and to estimate conditions under which solids separation fails (30,31). This method employs a series of batch settling tests to develop a relationship between zone settling velocity (ZSV) and initial total solids concentration (X_t). This data is then analyzed by regression techniques to determine a functional relationship between ZSV and X_t. These results are then used to compute the maximum solids flux that can be achieved in a continuous clarifier - thickener.

Batch flocculent settling tests are used to estimate total suspended solids concentrations in the effluent from a continuous clarifier-thickener. The test proceeds by adding a slurry to a settling column and then removing samples for suspended solids analysis at different times and depths during quiescent settling. Measured concentrations are plotted on a depth versus time graph and lines of equal concentration are estimated. This graph is then used in a standard procedure to predict effluent suspended solids concentrations as a function of overflow rate (32).

STOICHIOMETRY OF AUTOTROPHIC DENITRIFICATION

General Concepts of Microbial Stoichiometry

The transformations of compounds involved in microbial growth can be represented quantitatively by a balanced stoichiometric equation. The coefficients in such an equation can be used to determine the relationships among rates of reaction for all products and reactants.

$$\nu_a A + \nu_b B \rightarrow \nu_c C + \nu_d D \tag{8}$$

$$\frac{1}{\nu_a} R_a = \frac{1}{\nu_b} R_b = \frac{1}{\nu_c} R_c = \frac{1}{\nu_d} R_d \tag{9}$$

$\nu_a, \nu_b, \nu_c, \nu_d$ = stoichiometric coefficients for components A, B, C, and D, respectively;

R_a, R_b, R_c, R_d = rates of removal of components A and B and rates of production of C and D, respectively, [mg/ℓ·d].

The stoichiometry of microbial reactions in wastewater treatment has often been described in terms of observed yield coefficients, rather than the stoichiometric coefficients shown in Equation 8. An observed yield coefficient relates the production or removal of one component to the production or removal of another component. The component used as a reference is usually the pollutant of primary concern in the treatment process. For example, the primary goal in the treatment of sewage is the removal of oxygen-demanding organics measured as chemical (COD) or biochemical (BOD) oxygen demand. A major problem of these systems is handling the excess biomass produced. The stoichiometric relationship between these two concerns is usually expressed as an observed biomass yield coefficient that relates the amount of biomass produced per mass of COD or BOD removed. In general, the observed yield coefficient for some component C using component A as reference can be defined as follows.

$$Y_{c_{obs}} = \frac{\nu_c \text{ (molecular weight of C)}}{\nu_a \text{ (molecular weight of A)}} \tag{10}$$

$Y_{c_{obs}}$ = observed yield coefficient for component C using component A as reference, [mg/mg].

The equation representing the observed stoichiometry of microbial growth can be considered to be a linear combination of two subordinate equations (33). These subordinate equations represent the two basic processes involved in microbial growth--energy conversion and cell synthesis. Simple element and charge balances are used to construct the sub-reactions. In all but photosynthetic growth both sub-reactions are oxidation-reduction reactions. Therefore, it is convenient to express them on a basis of a one electron transfer to facilitate construction of the overall reaction.

$$\text{Observed Reaction} = f_e\ (\text{Energy Reaction}) + f_s\ (\text{Synthesis Reaction})$$

f_e, f_s = fraction of electron equivalents in observed reaction allocated to energy and synthesis sub-reactions, respectively. (11)

$$f_e + f_s = 1.0 \tag{12}$$

Microbial growth does not normally display a constant stoichiometry. This is due to variations in the composition of cell material and differences in the efficiency with which the microbes couple energy transformation with cell synthesis. The manner in which these processes are coupled depends on environmental variables and is expressed in the values of f_e and f_s. A high efficiency (high values of f_s) occurs at maximum growth rates when growth conditions are optimal. As growth rate declines, a smaller fraction of the energy made available in the energy reaction is effectively used to produce biomass.

The growth rate of microorganisms in a biological waste treatment system is the primary variable related to process performance, so it is easy to relate changes in stoichiometry to process operations (34). The growth rate in these systems is usually expressed indirectly through the operational variable called the solids residence time (θ_c). This variable is equal to the reciprocal of the growth rate, and is calculated by dividing the total amount of biomass in the system by the amount removed per unit time (34).

Several reports on the effect of growth rate on microbial stoichiometry are available (35-39). In most instances the effect of θ_c on one stoichiometric parameter is reported. Since the observed stoichiometric equation is a linear combination of two other balanced equations, specifying any one stoichiometric parameter determines all others. The parameter most often used to express stoichiometry in biological wastewater treatment is the observed biomass yield (Y_{obs}), which relates the amount of biomass produced per substrate utilized. In most instances Y_{obs} decreases with increasing θ_c.

Microbial stoichiometry can also be used to calculate oxygen uptake rates (38), and efficiencies of nitrogen and phosphorus removal in wastewater treatment systems (40). In microbial processes such as nitrification and denitrification, where hydrogen ions are produced or destroyed, the stoichiometric equation can be used to estimate process performance from

alkalinity measurements.

Thermodynamic Based Predictions of Microbial Stoichiometry

A method has been developed by McCarty to predict microbial stoichiometry by estimating f_e and f_s using theoretical arguments (41). The basis of the method is a balance on the primary energy storage component of the cell, adenosine triphosphate (ATP). Most reactions which release energy produce ATP. Most reactions which require energy consume ATP.

$$\text{ATP-energy produced by cell} = \text{ATP-energy used by cell} \qquad (13)$$

Estimates of the ATP-energy produced by the cell are made from thermodynamic analysis of the energy available from the energy reaction. The change in Gibbs Free Energy (ΔG) for a reaction is the best measure of the available energy released or required by that reaction. The value of ΔG for any given reaction will vary with changing environmental conditions such as temperature, pH, and relative amounts of reactants and products. For the range of these parameters usually encountered in microbial systems, the variation in ΔG is small. Therefore, a value of ΔG measured under standard conditions (ΔG^o) is used in these calculations.

Microorganisms are not completely efficient in producing or in utilizing ATP. Therefore, a factor representing the efficiency of energy conversion must be used to determine ATP-energy from thermodynamic energy. This efficiency factor could vary with changing environmental factors and could be different for the energy production and utilization processes. However, in this analysis it will be considered constant. A value for k of 0.6 has been found to be the best estimate for the energy efficiency of a variety of microorganisms (41). The ATP-energy balance incorporating k is:

$$f_e k \left[\begin{array}{l}\text{change in Gibbs Free}\\ \text{Energy for one elec-}\\ \text{tron equivalent of}\\ \text{energy reaction}\end{array}\right] = f_s \left[\begin{array}{l}\text{ATP-energy required}\\ \text{for synthesis of one}\\ \text{electron equivalent}\\ \text{of cells}\end{array}\right] \qquad (14)$$

k = efficiency factor for microbial energy conversion.

The energy requirement for synthesis is estimated from experimental data. Several different microorganisms have been found to require approximately 7.5 kilocalories of ATP-energy to produce one electron equivalent of cells from appropriate biosynthetic intermediates (41). Pyruvate was chosen as the synthesis intermediate for this ATP-energy balance because it appears in both biosynthetic and catabolic pathways in several microorganisms (41). The ATP-energy required for synthesis consists of the sum of the ATP-energy necessary to convert the carbon source to pyruvate plus that required to convert pyruvate to cell material. Conversion of the carbon source to pyruvate will sometimes release ATP-energy. In this case it should be subtracted from the ATP-energy necessary to convert pyruvate to cell material to obtain the ATP-energy required for synthesis. If a nitrogen source is

used by the microorganism which is not at the oxidation level of ammonia, the energy required to transform it to that level must also be included in calculating the energy required for synthesis.

$$f_e k(-\Delta G_e^o) = f_s\left(\frac{\Delta G_p^o}{k^m} + \Delta G_c^o\right), \quad m = \begin{cases} +1 & \Delta G_p^o > 0 \\ -1 & \Delta G_p^o < 0 \end{cases} \tag{15}$$

ΔG_e^o = standard free energy change for oxidation of one electron equivalent by energy reaction, [kcal/electron equivalent];

ΔG_p^o = standard free energy change for conversion of one electron equivalent of carbon source to pyruvate, [kcal/electron equivalent];

ΔG_c^o = ATP-energy required to convert one electron equivalent of pyruvate to cell material,

= 7.5 [kcal/electron equivalent];

m = coefficient indicating whether energy is released (-1) or used (+1) by reaction which converts carbon source to pyruvate.

Solving Equation 15 for the ratio f_e/f_s gives:

$$f_e/f_s = \frac{\frac{\Delta G_p^o}{k^m} + \Delta G_c^o}{k(-\Delta G_e^o)} \tag{16}$$

Individual values for f_e and f_s can be calculated by noting that they are fractions of a whole.

$$f_e/f_s = \frac{1}{f_s} - 1 \tag{17}$$

Theoretical Prediction of Autotrophic Denitrification Stoichiometry

A slight modification of McCarty's method is necessary to apply it to autotrophic denitrification. Thiobacillus denitrificans reduces carbon dioxide to cell mass in the same manner as photosynthetic cells. It has been shown that, from an energetics viewpoint, it is best to assume that water is the electron donor for this reduction even in non-photosynthetic cells (41). Photosynthetic cells excrete oxygen as a by-product of this reaction, but this cannot occur in autotrophic denitrification because it is an anaerobic process. Thus, oxygen produced in the synthesis reaction during autotrophic denitrification must be reduced by electrons from the energy reaction. Figure 4 shows a schematic of the proposed electron flow in autotrophic denitrification. In the overall reaction stoichiometry, sulfur

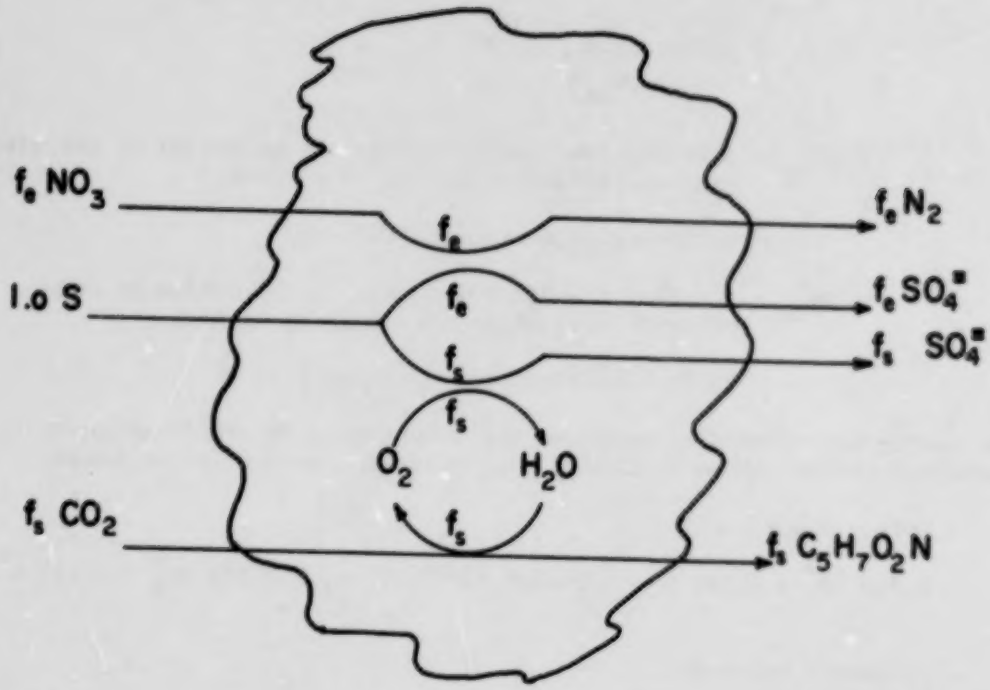


Figure 4. Proposed electron flow for autotrophic denitrification under anaerobic conditions with water the electron donor for synthesis.

is the apparent electron donor for synthesis rather than water, since internal recycle excludes oxygen from the overall stoichiometry.

The balance on ATP-energy for autotrophic denitrification using the above assumptions is:

$$\text{ATP-energy produced by cell} = \text{ATP-energy used by cell} \tag{13}$$

$$k[f_e(-\Delta G^o_{sn}) + f_s(-\Delta G^o_{so})] = f_s\left[\frac{\Delta G^o_p}{k} + \Delta G^o_c\right] \tag{18}$$

$$f_e/f_s = \frac{\Delta G^o_p/k + \Delta G^o_c + k\Delta G^o_{so}}{k(-\Delta G^o_{sn})} \tag{19}$$

ΔG^o_{sn} = standard free energy change for oxidation of one electron equivalent of sulfur by nitrate;

= 21.78 kcal/electron equivalent;

ΔG^o_{so} = standard free energy change for oxidation of one electron equivalent of sulfur by oxygen;

= 23.33 kcal/electron equivalent.

The energy and synthesis reactions for autotrophic denitrification using elemental sulfur can be expressed on a one electron equivalent basis.

Energy Reaction

$$0.200\ NO_3^- + 0.167\ S + 0.0667\ H_2O \rightarrow 0.100\ N_2 + 0.167\ SO_4^= + 0.133\ H^+ \tag{20}$$

Synthesis Reaction

$$0.250\ CO_2 + 0.167\ S + 0.050\ NH_4^+ + 0.267\ H_2O \rightarrow 0.050\ C_5H_7O_2N + 0.167\ SO_4^= + 0.383\ H^+ \tag{21}$$

These reactions and Equation 11 can be used to calculate Y_{obs} from the ratio f_s/f_e. This stoichiometric yield coefficient relates the amount of biomass, measured as organic nitrogen, produced per mass of nitrate-nitrogen removed.

$$Y_{obs} = \frac{f_s(1/20)(14)}{f_e(1/5)(14)} = \frac{f_s}{f_e}(0.25) \tag{22}$$

An observed yield coefficient of 0.084 mg organic-N/mg NO_3^--N can be calculated using Equations 19 and 22 and a value for k of 0.6.

Using a similar analysis balanced stoichiometric expressions can be

derived for other sulfur electron donors. For example, Equations 23 and 24 represent the balanced stoichiometry for thiosulfate and sulfide, respectively.

Thiosulfate

$$0.844\ S_2O_3^{=} + NO_3^- + 0.347\ CO_2 + 0.0865\ HCO_3^- + 0.0865\ NH_4^+ + 0.434\ H_2O \rightarrow 1.689\ SO_4^{=} + 0.5\ N_2 + 0.0865\ C_5H_7O_2N + 0.697\ H^+ \quad (23)$$

Sulfide

$$0.422\ H_2S + 0.422\ HS^- + NO_3^- + 0.347\ CO_2 + 0.865\ HCO_3^- + 0.0865\ NH_4^+ \rightarrow 0.844\ SO_4^{=} + 0.5\ N_2 + 0.0865\ C_5H_7O_2N + 0.409\ H^+ \quad (24)$$

When soluble electron donors such as sulfide or thiosulfate are employed two additional stoichiometric parameters become useful. The consumptive ratio, C_R, and the feed ratio, C_F, are defined in Equations 25 and 26, respectively.

$$C_R = \frac{\text{electron equivalents of } S_2O_3^{=} \text{ or } S^{=} \text{ consumed}}{\text{electron equivalents of } NO_3^- \text{ reduced}} \quad (25)$$

$$C_F = \frac{\text{electron equivalents of electron donor (sulfur compound) in feed}}{\text{electron equivalents of electron acceptor (nitrate) in feed}} \quad (26)$$

C_F and C_R are defined such that if C_F is greater than C_R, then growth will be nitrate limiting. When C_F is less than C_R growth will be electron donor (sulfur compound) limiting.

SECTION 6

ANALYTICAL TECHNIQUES

Table 2 presents a summary of the analytical techniques employed in this project. Detailed discussion of analytical techniques in the subsequent paragraphs of this section is restricted to those methods that were non-routine and required developmental effort by the project staff.

A modification to the cadmium reduction method for nitrate (42) was required. To standardize flow rates, a modified cadmium reduction column was constructed by filling a length of 6.3 mm glass tubing with 0.25-0.42 mm cadmium metal filings to a depth of approximately 80 mm, as shown schematically in Figure 5. This reductor was connected to another piece of glass tubing which was bent to facilitate sample collection and the two were placed in a buret holder on a ring stand. The top of the reductor was connected to a glass funnel (maximum diameter 100 mm) held on the ring stand approximately 500 mm above the cadmium. All connections in this apparatus were made with clear plastic tubing. Samples were prepared for analysis in the standard manner (42). The height of the funnel above the reductor maintained relatively constant flow rates during an analysis. The column was standardized on a regular basis by passing a sample of known nitrate concentration. Corrections for the reagent blank, column blank and partial reduction of nitrite in the column were made when calculating the nitrate concentration. Experience showed that 10 percent of the nitrate that was applied to the column would be reduced to some nitrogen compound other than nitrite.

$$N = (D.F.)\ \frac{N_s}{A_s - A_{bl}}\ [(A_n - A_{cb}) - (A_{ni} - A_{bl})\ (.9)] \qquad (27)$$

N = nitrate-nitrogen concentration in sample, [mg/ℓ];

N_s = nitrate-nitrogen concentration in standard, [mg/ℓ];

D.F. = dilution factor, i.e., volume of diluted sample divided by volume of original sample;

A_n = absorbance of diluted sample passed through column;

A_s = absorbance of standard;

A_{cb} = absorbance of column blank, i.e., blank sample passed

TABLE 2. SUMMARY OF ANALYTICAL TECHNIQUES

Analysis	Method	Ref.	Comments
Nitrogen Species			
Nitrate	cadmium reduction	42	see text
Nitrite	diazotization	42	
Organic Nitrogen	digestion and 1) distillation and acidometric titration 2) ammonia probe	43	modified for micro-kjeldahl analysis
Nitrogenous Gases	gas chromatography		see text
Sulfur Species			
Elemental Sulfur	iodometric titration		see text
Sulfate	turbidometric	42	
Thiosulfate	iodometric	43	
Sulfide	titrimetric	43	
Other Analysis			
ATP	luciferin-luciferase assay	44	see Appendix A2
Alkalinity	Gran acidometric titration	45	see text
pH	pH meter with glass electrode		Accumet 320 pH meter (Fisher Sci. Co.)
COD	dichromate digestion	43	dilute reagents
Total Suspended Solids	gravimetric	43	glass fiber filters (Whatman GF/C)

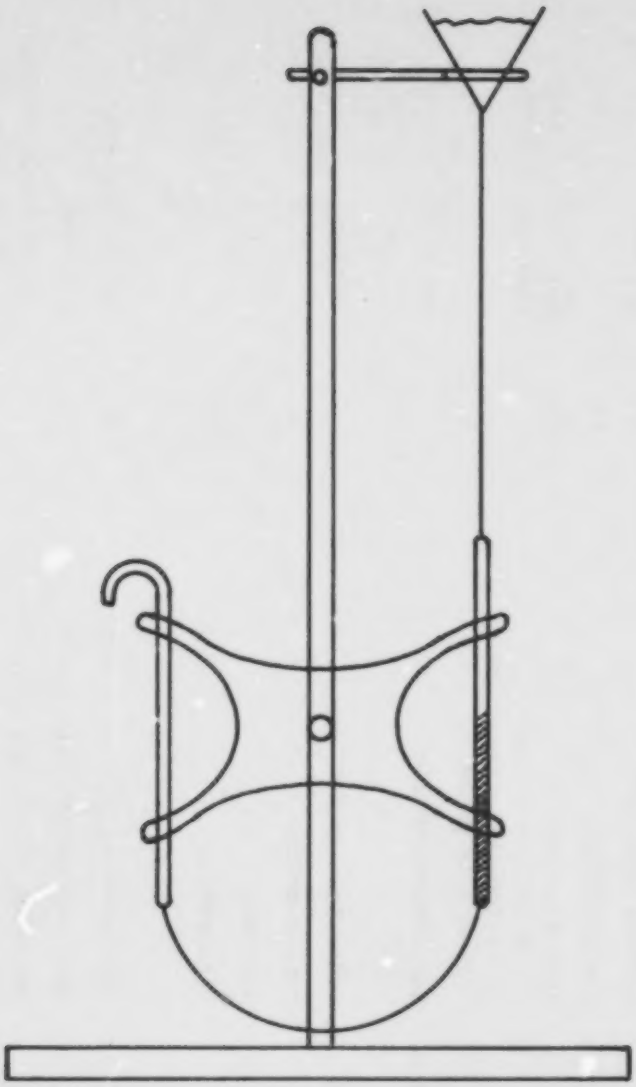


Figure 5. Schematic of cadmium reduction column used for nitrate analysis.

through reduction column;

A_{ni} = absorbance of diluted sample not passed through column, i.e., absorbance due to nitrite;

A_{bl} = absorbance of reagent blank.

0.9 = empirically determined efficiency of reduction of nitrate to nitrite.

Organic nitrogen was measured by sulfuric acid digestion with mercury catalyst (43) followed by direct measurement of ammonia with an ammonia probe (Orion Model 95-10) or distillation and titration of the ammonia (43). In the initial period of this study, the probe method was used exclusively. However, this technique developed erratic and insensitive behavior and was replaced by the distillation and titration procedure.

Elemental nitrogen, nitrous oxide, nitric oxide, carbon dioxide and oxygen were separated and measured in a two-column gas chromatograph (Varian, Model 90-P3) with thermal conductivity detector. The first column was packed with Poropak Q, 0.15-0.18 mm (80/100 mesh), the second with molecular sieve 13 x, 0.25 - 0.60 mm (30/60 mesh).

A procedure was developed to analyze elemental sulfur in aqueous solution (Appendix A1). The procedure consisted of converting the sulfur to thiosulfate by boiling with a sulfite solution, then analyzing the thiosulfate by an iodine titration after complexing residual sulfate with formaldehyde.

High, variable concentrations of carbon dioxide in the reactors caused variable endpoints in alkalinity titrations. This problem was overcome by using a Gran titration method for alkalinity which determines the endpoint from titration data (45). A material balance on hydrogen ions is made by assuming that all H^+ added after the equivalence point is passed, remains in solution as the free ion.

$$(V_s - V_t)\ 10^{-pH} = (V_t - V_t^e)\ C_a \qquad (28)$$

V_s = volume of sample, [mℓ];

V_t = volume titrated, [mℓ];

V_t^e = volume titrated at equivalence point, [mℓ];

C_a = concentration of acid in titrant, $[\frac{\text{equivalents}}{\text{mℓ}}]$.

Volume titrated and pH were recorded at four points below pH = 4.8 and a least squares regression performed to determine V_t^e .

SECTION 7

EXPERIMENTAL STUDIES

The experimental aspects of this project were conducted in three phases. In the first, and most extensive phase (Phase I) elemental sulfur was employed in both semicontinuous and continuous flow complete mix slurry-type denitrification reactors. The second experimental phase (Phase II) employed thiosulfate and/or sulfide in completely mixed, semicontinuous flow denitrification reactors. The final experimental phase (Phase III) employed sulfur in continuous flow packed bed denitrification reactors. The procedures, results, and discussion of each phase of the experimental study are presented separately in subsequent subdivisions of this section.

PHASE I - SLURRY REACTORS USING ELEMENTAL SULFUR

A two-part experimental plan was developed to investigate the characteristics of autotrophic denitrification in slurry reactors fed elemental sulfur. The first part of the plan was to develop and characterize a microbial enrichment culture that could denitrify with elemental sulfur. In the second part, these cultures were used in continuous culture experiments to determine the kinetic and stoichiometric behavior of the process. Solids separation characteristics of sulfur-biomass slurries from these continuous cultures were also evaluated. Throughout Phase I, the concentration of biomass in the reactors which is denoted by the symbol X was estimated by measuring the mixed liquor non-filterable organic nitrogen. Thus, in all presentations of results and expression of the ratio S/X, the units of X are mg/ℓ organic-N. It was necessary to use suspended organic nitrogen as a surrogate parameter for biomass because the high concentrations of inorganic sulfur contained in the slurry reactors rendered determination of biomass by the conventional surrogate, i.e., volatile suspended solids, a non-reproducible and highly inaccurate exercise. In some of the continuous flow experiments conducted during this phase of the experimental study, ATP measurements were performed on the mixed liquor suspended solids. While such measurements are considered to be correlatable to active bacterial biomass, it was felt that determination of suspended organic nitrogen was a more reproducible measurement and more easily related to actual bacterial biomass through the empirical formula widely used to chemically describe bacterial protoplasm, i.e., $C_5H_7O_2N$.

Culture Characterization Experiments

Experimental Plan--

The culture characterization portion of Phase I was primarily concerned

with estimating process performance so that the continuous culture experiments could be more efficiently executed. The semicontinuous reactors used during these experiments were operated by periodically removing waste solids and adding elemental sulfur and a media consisting of tap water enriched with nitrate and nutrients. One series of experiments estimated the effect of sulfur feed rate on the kinetics of autotrophic denitrification by varying the amount of sulfur added to several semicontinuous reactors. Another series of reactors was operated with varying amounts of ammonia in the feed to determine if ammonia was strictly required as the nitrogen source for cell synthesis, as reported for pure cultures of Thiobacillus denitrificans. Temperature effects on the rate were estimated by operating two reactors at different temperatures. Three other batch experiments were conducted to determine reaction stoichiometry. Table 3 summarizes the experimental plan during culture characterization.

TABLE 3. SUMMARY OF EXPERIMENTAL PLAN

Effect Measured	Magnitude of Variable in Experiment
Sulfur feed ratio	5, 25, 100, 500 (mg S/mg NO_3^--N)
Ammonia feed ratio	0, 0.05, 0.10, 0.20 (mg NH_4^+-N/mg NO_3^--N)
Temperature	12, 20 (^oC)
Stoichiometry	(3 replicate experiments)

Experimental Techniques --

Figure 6 shows a schematic representation of the semicontinuous reactor system used during culture characterization experiments. These reactors were one-liter, glass bottles mixed by magnetic stirrers. The bottles were sealed with a rubber stopper and the gas produced during denitrification was collected in a graduated cylinder inverted in a beaker of water. Reactor temperature was controlled by placing the reactors in constant temperature rooms. All experiments were conducted at 20°C except for one reactor operated at 12°C to estimate temperature effects.

Initial seed for this study was obtained from samples of soil, mud, and anaerobically digested sludge from a municipal wastewater treatment plant. Approximately 80 grams of each sample were placed in 300 mℓ glass bottles to which standard media containing thiosulfate instead of sulfur was added. The bottles were capped and incubated anaerobically at room temperature. All samples showed increased turbidity after several weeks, but the digested sludge sample from a nitrifying activated sludge plant was most active. Supernatants from all the bottles were collected, and mixed together. This culture was regularly fed standard media and was adapted to growth on elemental sulfur before being used as the seed for all the reactors used throughout the entire study.

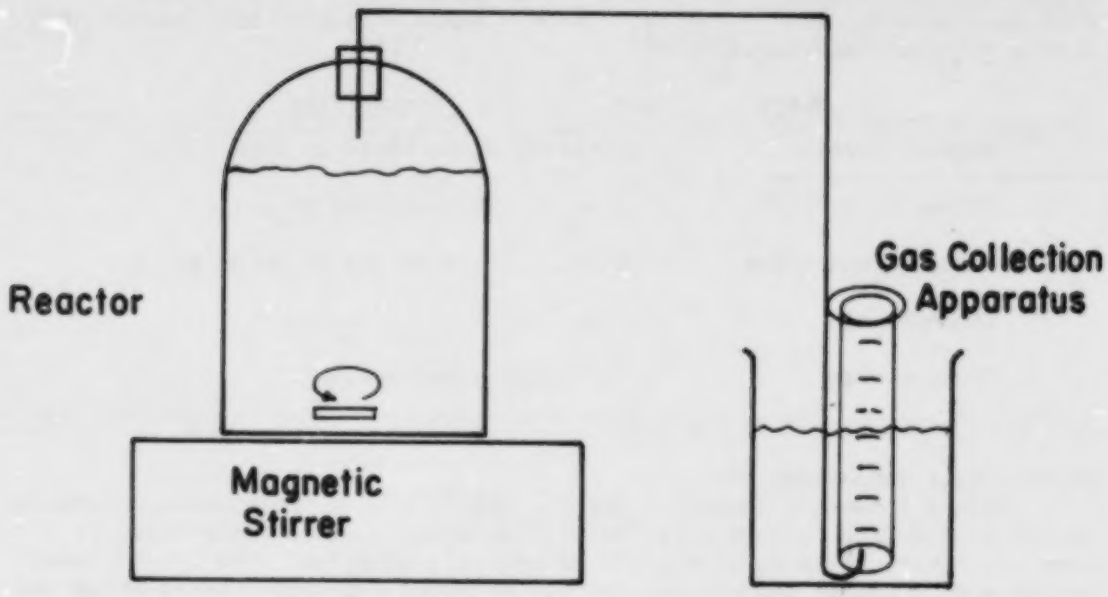


Figure 6. Schematic of semicontinuous culture reactor system.

The semicontinuous reactors were operated at a hydraulic retention time of 5 days and a solids retention time of 20 days by wasting mixed liquor and supernatant and feeding a nutrient solution every 4 days. At each feeding, reactor walls were scraped; 200 mℓ of the reactor contents were wasted; and, the solids were allowed to settle. 600 mℓ of clear supernatant were then removed and 800 mℓ of feed solution were added. Elemental sulfur was dried and passed through a 150 micron sieve before addition to the reactor. Gas production was measured at various times after feeding and the rate determined from the slope of the cumulative gas volume-time curve. The rate of gas production was used to measure the rate of denitrification, since the gas being produced was almost entirely elemental nitrogen formed by microbial reduction of nitrate. The standard media used in the semicontinuous reactors was a modification of the media used by Baalsrud (1). Table 4 lists the components of this media. Tap water that had been dechlorinated by overnight aeration was used as the basis of the media.

TABLE 4. COMPOSITION OF MEDIA FOR SEMICONTINUOUS CULTURE EXPERIMENTS

Constituent	Concentration (milligrams/liter)
S^o	2,500
KNO_3	721 (100 mg/ℓ as N)
KH_2PO_4	300
K_2HPO_4	1,500
$NaHCO_3$	1,000
NH_4Cl	76 (20 mg/ℓ as N)
$MgCl_2 \cdot 6H_2O$	500
$FeCl_3 \cdot 6H_2O$	10

Experimental Results--

Variable sulfur to nitrate-nitrogen feed ratio -- A major element in determining the feasibility of autotrophic denitrification using elemental sulfur was to evaluate how the amount of sulfur available to the microorganisms affected their rate of denitrification. This characteristic was measured in a series of experiments in which four semicontinuous reactors were operated as described above except that different amounts of sulfur were added to each. Table 5 and Figure 7 show the results of these experiments.

Variable ammonia-nitrogen to nitrate-nitrogen feed ratio -- The effect of ammonia on the rate of denitrification was measured by operating a series

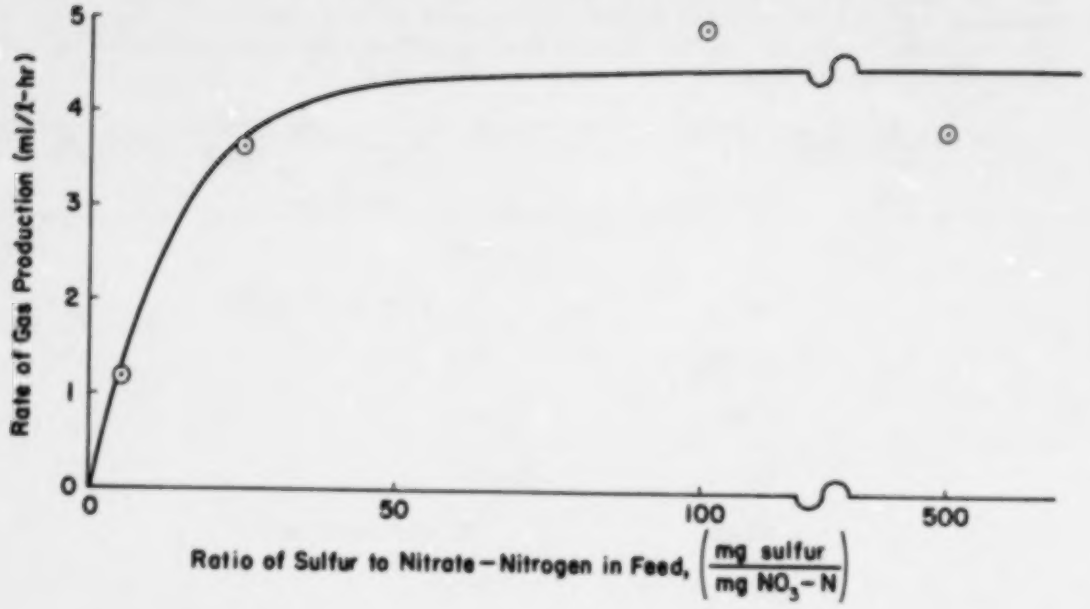


Figure 7. Rate of gas production versus sulfur to nitrate-nitrogen feed ratio.

TABLE 5. EFFECT OF SULFUR TO NITRATE FEED RATIO ON GAS PRODUCTION RATE

Expt. No.	Nitrogen Gas Production Rate (mℓ/ℓ · hr) S/NO_3^--N			
	5	25	100	500
1	0.7	3.8	3.7	3.6
2	1.1	3.5	3.6	3.5
3	1.3	3.6	5.6	4.9
4	1.5	3.5	5.4	3.1
AVG.	1.2	3.6	4.9	3.9

of semicontinuous reactors as previously described except that the amount of ammonia in the feed to each reactor was different. Feed ratios of 0, 0.5, 0.10 and 0.20 (mg NH_3-N/mg NO_3^--N) were used. The results are tabulated in Table 6 and presented graphically in Figure 8.

TABLE 6. EFFECT OF AMMONIA TO NITRATE FEED RATIO ON GAS PRODUCTION RATE

Expt. No.	Nitrogen Gas Production Rate (mℓ/ℓ · hr) NH_3-N/NO_3^--N			
	0.0	0.05	0.10	0.20
1	1.7	2.4	--	3.8
2	2.8	3.2	--	3.5
3	2.5	2.5	--	3.6
4	3.0	---	3.3	3.6
AVG.	2.5	2.7	3.3	3.6

Variable temperature study -- A temperature dependence study was performed with semicontinuous reactors operated at 12 and 20°C. Operation of

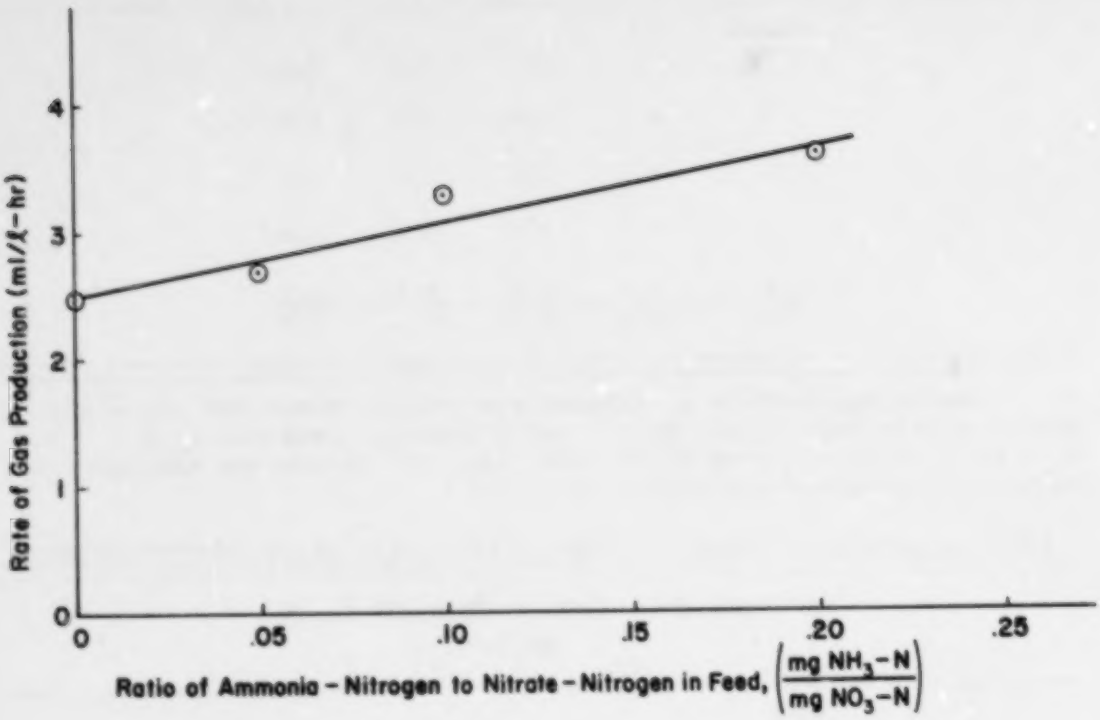


Figure 8. Rate of gas production versus ammonia-nitrogen to nitrate-nitrogen feed ratio.

a reactor at 30°C was attempted but could not be sustained. Gas production was initially rapid at 30°C but after a few weeks of operation it decreased to very low levels. Nitrite-nitrogen accumulated in this reactor to a level nearly equal to the nitrate-nitrogen concentration of the feed solution. Table 7 presents the results obtained from the reactors operated at 12 and 20°C. Although the danger of fitting a curve to two data points is acknowledged, quantitative measures of temperature dependence are useful. Therefore, an Arrhenius activation energy of 13.0 kcal/mole was calculated from the average gas production rates (Table 7) using Equation 7.

TABLE 7. EFFECT OF TEMPERATURE ON GAS PRODUCTION RATE

	Gas Production Rate (ml/l · hr)	
Expt. No.	12°C	20°C
1	1.0	2.8
2	0.9	2.7
3	---	2.7
4	1.1	---
5	1.4	2.6
6	1.4	2.8
7	1.4	---
8	1.5	---
AVG.	1.2	2.7

Batch stoichiometry -- An estimate of reaction stoichiometry was desired for the preliminary characterization of autotrophic denitrification using elemental sulfur. This data was obtained in a series of batch experiments conducted by taking a seed obtained from the semicontinuous reactors and mixing it with standard media. When gas production ceased, the reactor was spiked with a feed solution ten times the concentration of standard media. This procedure was repeated several times to produce a sufficient amount of biomass to insure accurate measurement. Organic nitrogen, nitrate, nitrite, sulfate and alkalinity were measured in the initial and final reactor media and in the concentrated feed solution. Table 8 presents the results of these experiments expressed as observed yield coefficients. Observed yield coefficients for biomass (Y_{obs}), sulfate-sulfur ($Y^{SO_4-S}_{obs}$), and alkalinity (Y^{Alk}_{obs}) were calculated by dividing the amount of each compound produced or destroyed in the microbial reaction by the amount of nitrate-nitrogen removed by the microorganisms. Since these coefficients are based on measurements made at the end of each experiment, they are average values

and cannot represent possible variations in the values of the coefficients during the course of the experiment.

TABLE 8. BATCH STOICHIOMETRIC COEFFICIENTS

Expt. No.	Y_{obs} ($\frac{mg\ organic\text{-}N}{mg\ NO_3^-\text{-}N}$)	$SO_4^=\text{-}S$ ($\frac{mg\ SO_4^=\text{-}S}{mg\ NO_3^-\text{-}N}$)	Y_{obs}^{Alk} ($\frac{meq}{mg\ NO_3^-\text{-}N}$)	$\frac{Y_{obs}^{SO_4\text{-}S}}{Y_{obs}^{Alk}}$ ($\frac{mg\ SO_4^=\text{-}S}{meq}$)
1	0.096	2.27	0.088	25.8
2	0.075	2.29	0.120	19.1
3	0.095	2.49	0.132	18.9
AVG.	0.089	2.35	0.113	21.3

Continuous Culture Experiments

Experimental Plan--

Two series of continuous culture experiments were conducted to delineate the kinetics and stoichiometry of autotrophic denitrification at 20°C. Five reactors were operated at different solids retention times in the first series, to determine the effect of θ_c on steady state reaction stoichiometry and nitrate removal. The effect of mixed liquor S/X was evaluated in an additional series of four reactors. The parameter, S/X, of the mixed liquor was defined as the ratio of suspended elemental sulfur concentration to the suspended organic nitrogen concentration. After obtaining steady state data from this second series of reactors, transient rate tests were performed to determine the maximum attainable unit rate of denitrification for each of the four values of S/X studied. One continuous reactor was also operated at each of two other temperatures to obtain steady state and transient kinetic data.

In addition to the kinetic studies, a series of zone settling tests was performed on sulfur-biomass slurries from continuous cultures operated at three different values of S/X. Data from these tests were used in a batch flux analysis of the settling properties of the slurries. The relationship between effluent suspended solids and clarifier overflow rate was estimated from data taken during flocculent settling tests conducted with the same three slurries. An additional flocculent settling test was performed to determine the effect of initial solids concentration. Table 9 summarizes the experimental plan followed during the continuous culture experiments.

Experimental Techniques--

Reactor operations--Figure 9 shows a schematic representation of the continuous culture reactor system. Six-liter, conical, glass reactors with a two-liter glass inner cone and a 45 x 255 mm settling cylinder were used (Biooxidation System, Horizon Ecology Company, Chicago, Illinois). Mixing

TABLE 9. SUMMARY OF EXPERIMENTAL PLAN FOR CONTINUOUS CULTURE EXPERIMENTS

Experiment	S/X (mg S/mg org-N)	θ_c (days)	Temperature (°C)	Transient Microbial Assimilation Tests	Settling Tests
Variable θ_c					
1*	∿ 145	10	20	x	
2	∿ 145	15	20	x	
3	∿ 145	20	20		
4	∿ 145	25	20		
5	∿ 145	30	20		x
Variable S/X					
1	45	near	20	x	x
2	56	maximum	20	x	x
3	100	attainable	20	x	
4*	142	value	20	x	
5	194		20	x	
Variable T					
1	145	near	12	x	
2*	145	maximum	21	x	
3	145	attainable value	30	x	

* Indicates single experiment used to describe effect of several variables.

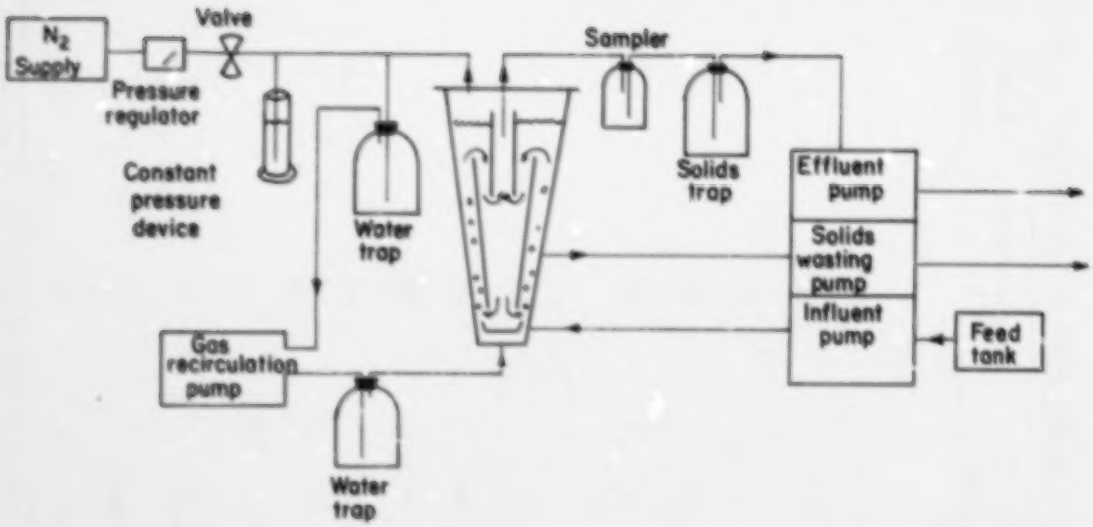


Figure 9. Schematic of continuous culture reactor system.

was accomplished by a recirculation pump which supplied nitrogen gas to the reactors through fritted glass diffusers at a rate of about 0.3 m^3/min. The upward movement of the gas between the inner and outer cones caused an internal circulation pattern which kept solids in suspension and reactor contents well mixed. Solids were separated in the Plexiglass cylinder situated at the top of the inner cone. Clarified effluent was removed from the top of this cylinder and feed was supplied to the reactor by a peristaltic pump. Influent feed rate was maintained at approximately one liter per hour which set the clarifier overflow rate at 15 $m^3/m^2 \cdot d$. The feed system for the continuous reactors consisted of a peristaltic pump with different heads for influent and effluent lines, a 110 liter polyethylene feed tank, and connecting lengths of clear plastic tubing.

Certain modifications were required to adapt the purchased reactors for autotrophic denitrification experiments. Anaerobic conditions were maintained within a reactor by fitting it with a Plexiglass cover sealed with a rubber O-ring. To minimize atmospheric oxygen leaks, a positive gas pressure was maintained within the reactor. This was done by connecting a pressurized tank of dry nitrogen to the reactor through a pressure regulator and constant pressure device. Recycling nitrogen gas within the reactor caused an accumulation of carbon dioxide in the gas stream which equilibrated at a level of about 2-4 percent. High settling velocities of the sulfur-biomass particles caused solids to accumulate at the bottom sides of the reactors. This dead area was eliminated by installing plastic funnels shaped to fit the reactor bottom. Other dead areas around glass tubing connections to the reactor were eliminated by installation of rubber plugs. Two traps on the effluent line were used to take samples and return solids lost from the reactor. The possibility of microbial growth in the lines from the reactor to the feed tanks was decreased by the addition of an air-break between the reactor and the feed pump.

Microbial seed for the continuous cultures was obtained from the semi-continuous reactors. Biomass concentration was increased by initially feeding standard media containing thiosulfate instead of sulfur. When the desired biomass concentration was attained, the cultures were acclimated to elemental sulfur and continuous flow was begun. Wasting from the reactors was done once a day according to the following formula:

$$V_w = \frac{V \, \Delta t}{\theta_c} \tag{29}$$

V = volume of reactor, [ℓ];

V_w = volume of mixed liquor wasted, [ℓ];

Δt = time interval between wasting, [d].

Each week the reactors were cleaned by turning off all pumps, scraping the reactor walls and scouring the inner cone and settling cylinder. Feed lines were changed and cleaned with a 5 percent solution of sodium

hypochlorite. Each day the general condition of the reactors were noted and the volumes of feed solution in the feed tanks were recorded. The volumetric flow rate used in calculating the hydraulic retention time (θ) was determined by dividing the change in feed solution volume by the time interval between measurements. Influent flow rates were adjusted each day if necessary to maintain constant flows and sufficient sulfur was added to maintain the desired value of S/X.

Measurement of pH and a spot test for nitrite were performed daily on effluent samples from each reactor. The spot test for nitrite was considered an adequate measure of a reactor's performance because nitrate was never present in significant amounts in the absence of nitrite. Weekly analyses for alkalinity were made to check the reactors for oxygen leaks. A large decrease in effluent alkalinity would indicate a significant oxygen leak because the microorganism can use oxygen to oxidize sulfur (1). Since hydrogen ions would be produced by this oxidation (1), alkalinity would decrease in proportion to the amount of oxygen entering the reactors.

Composition of the feed for the continuous reactors was based on that used previously in a study of continuous culture heterotrophic denitrification (46). Table 10 lists the nutrients added to supplement dechlorinated tap water. Laboratory-grade, resublimed sulfur (Fisher Chemical Company) was dried at 60°C and passed through a 150 micron sieve before addition to the reactors. Sulfur particle size distribution was estimated by microscopic analysis of 200 particles which had been dried and passed through a 74 micron sieve. A mean average dimension of 82 microns was obtained with 10 and 90 percentile points being 42 and 123 microns, respectively. Since particles larger than 74 microns were observed, it can be concluded that some agglomeration of sulfur particles occurred.

Steady state techniques--Steady state data were gathered during a sampling period of at least three days which began only after the reactor had been operating for a period at least as long as three times the solids retention time. Nitrite and alkalinity were analyzed immediately after the effluent samples were taken and filtered. Filtration was done with 0.45 micron membrane filters (HAWP, Millipore Company) and a glass fiber prefilter (GF/C, Whatman) which had been washed with 240 ml of distilled-deionized water. Sulfate and nitrate analyses were performed on filtered effluent samples after storage at -10°C. Previous experience showed that filtration and cold storage was an effective means of preservation for nitrate and sulfate. Samples of mixed liquor were taken from reactor wastage and analyzed for elemental sulfur and total kjeldahl nitrogen. Organic nitrogen was equivalent to total kjeldahl nitrogen for these samples since ammonia concentrations were negligible. Organic nitrogen was used as a measure of biomass in this study, since elemental sulfur interfered with gravimetric analysis of suspended solids or volatile suspended solids.

Since samples were taken from the wastage, the measured values of sulfur and organic nitrogen were adjusted to better represent average reactor concentration before and after wastage. Calculation of average reactor

TABLE 10. COMPOSITION OF CONTINUOUS CULTURE FEED SOLUTION

Constituent	Concentration
KNO_3	30 mg/ℓ as N (216 mg/ℓ KNO_3)
NH_4Cl	1.5 mg/ℓ as N (6 mg/ℓ as NH_4Cl)
$NaHCO_3$	900 mg/ℓ
K_2HPO_4	10 mg/ℓ as P (56 mg/ℓ as K_2HPO_4)
$MgCl_2 \cdot 6H_2O$	1 mg/ℓ
$FeCl_3 \cdot 6H_2O$	1 mg/ℓ
$MnSO_4 \cdot H_2O$	1 mg/ℓ
$CaCl_2 \cdot 2H_2O$	1 mg/ℓ
pH	8.6

sulfur concentration also included consideration of the amount of sulfur added to the reactor.

$$X = \left(\frac{2V-V_w}{2V}\right)X_{wastage} \tag{30}$$

$$S = \left(\frac{2V-V_w}{2V}\right)S_{wastage} + \frac{S_{added}}{2V} \tag{31}$$

$X_{wastage}$, $S_{wastage}$ = biomass and sulfur concentration measured in wastage, [mg/ℓ]

S_{added} = amount of sulfur added, [mg].

Transient rate study techniques--Subsequent to steady state operation, duplicate transient rate tests were performed on some reactors to measure $U_{m,a}$ under conditions of an excess of nitrate. These reactors were spiked with a known amount of nitrate and samples for nitrate and nitrite analysis were taken at 30 minute intervals for 3-7 hours.

Since nitrate and nitrite are both microbially available electron acceptors during transient tests, a measure of their combined effect is

necessary. The concentration of equivalent nitrate-nitrogen was used for this purpose. This variable is equal to the concentration of nitrate-nitrogen in a solution without nitrite, which has the same concentration of electron equivalents as the solution of nitrate and nitrite in question. Solutions with equal amounts of electron acceptors are capable of oxidizing equal amounts of sulfur.

$$CEN = N + 0.6\ NI \tag{32}$$

CEN = equivalent nitrate-nitrogen concentration, [mg/ℓ]

N = nitrate-nitrogen concentration, [mg/ℓ]

NI = nitrite-nitrogen concentration, [mg/ℓ].

Data from the transient rate tests were analyzed by assuming that the rate of removal of equivalent nitrate is independent of its concentration. A non-steady state material balance equation was used to predict the equivalent nitrate concentration at any time for a given value of the rate constant.

$$Q(CEN)^{o} - Q(CEN) - V\frac{d(CEN)}{dt} + VK \tag{33}$$

$$CEN = CEN^{to}\ e^{-t/\theta} + (CEN^{o}-K\theta)(1-e^{-t/\theta}) \tag{34}$$

CEN^{o} = equivalent nitrate-nitrogen concentration in influent and in reactor at t = 0, respectively, [mg/ℓ],

K = rate constant for zeroth-order reaction $[\frac{mg}{\ell-d}]$.

The zeroth-order rate coefficient was determined from experimental data by choosing the value of K which minimized the sum of the squared differences between the measured equivalent nitrate-nitrogen concentration and the concentration predicted by Equation 34.

$$\min \sum_{i} [CEN_i - CEN^{to} e^{-t_i/\theta} - (CEN^{o}-K\theta)(1-e^{-t_i/\theta})]^2 \tag{35}$$

CEN_i = equivalent nitrate-nitrogen concentration measured in i^{th} sample, [mg/ℓ],

t_i = time after addition of nitrate solution when i^{th} sample taken; [d].

$U_{m,a}$ was then calculated using the average steady state biomass concentration.

$$U_{m,a} = \frac{K}{X} \tag{36}$$

Solids separation study techniques--Zone settling velocity tests were performed in one liter (60 x 355 mm) or two liter (80 x 385 nm) graduated cylinders. The settling velocity of a slurry was found to be unaffected by

the size of cylinder in which the settling test was conducted. An aluminum rod, bent at right angles every 6o mm was driven by a 1 rpm motor to provide stirring during the tests. Tests run without stirring, however, showed no difference in measured zone settling velocity (ZSV). Interface height was measured at 15 or 30 second intervals and ZSV calculated by dividing the average difference in height of the settling zone interface by the time interval between measurements. Samples were analyzed for total suspended solids after every test and used to calculate solids fluxes. Linear least squares regressions were performed on the data to determine the functional relationship between solids flux and solids concentration.

Flocculent settling tests were performed in a Plexiglass settling column (45 mm x 2.8 m) with sampling ports spaced at 0.2 m intervals. A test was begun by adding a slurry from a continuous culture to the top of the column and keeping it well mixed by a flow of nitrogen gas entering the bottom of the column. After the gas flow was discontinued and mixing currents had subsided, a stopwatch was activated. Samples for suspended solids analysis were taken at each port after the interface had passed and at several intervals thereafter. The time and liquid level height were recorded for each sample. This data was analyzed using a standardized procedure for flocculent settling tests (32).

Experimental Results--

Variable solids retention time--The effect of growth rate ($1/\theta_c$) on reaction kinetics was evaluated by operating five continuous reactors at the same sulfur feed rate and temperature but different values of θ_c. Solids retention times of 10, 15, 20, 25 and 30 days were evaluated.

Table 11 presents a summary of the results of these experiments. Organic nitrogen is used as a measure of biomass concentration in Table 11 as well as in all other presentations or discussions of experimental results in Phase I. Mixed liquor ATP concentration (X^{ATP}) is another indicator of biomass concentration. This parameter was measured during the variable θ_c experiments and results are shown in Table 11. ATP is the major compound for energy storage within a cell, so it is representative of the amount of biomass present, if the ratio of ATP to viable biomass remains constant. The stoichiometric parameters ΔSO_4-S and ΔAlk in Table 11 represent the increase in the effluent concentration of sulfate-sulfur and decrease in alkalinity respectively, relative to their influent concentration.

Graphical presentations of the dependence of Y_{obs} and X^{ATP}/X on θ_c are shown in Figures 10 and 11, respectively. Transient rate tests were performed on the 10 and 15-day reactors and that data is presented in Tables B1 and B2 in the Appendix. The recirculating gases within the reactors operated at θ_c = 25, 30 days were analyzed by gas chromatography. A trace of nitrous oxide (50 ppm) was detected but no nitric oxide was found.

Variable sulfur to biomass ratio--Four additional continuous culture reactors were operated at different values of S/X to evaluate this parameter's

TABLE 11. SUMMARY OF CONTINUOUS CULTURE RESULTS AT VARIOUS θ_c

Operating & kinetic parameters			Mixed liquor characteristics			Effluent characteristics		Stoichiometric Parameters		
θ_c (d)	θ (d)	S/X ($\frac{mg\ S}{mg\ N^*}$)	X ($\frac{mg\ N^*}{\ell}$)	X^{ATP} ($\frac{mg\ ATP}{\ell}$)	S ($\frac{g\ S}{\ell}$)	NO_3-N ($\frac{mg}{\ell}$)	NO_2-N ($\frac{mg}{\ell}$)	Y_{obs} ($\frac{mg\ N^*}{mg\ N^{**}}$)	ΔSO_4-S ($\frac{mg\ S}{\ell}$)	ΔAlk ($\frac{meq}{\ell}$)
10	0.25	142	83 (10)	1.05 (0.15)	11.8 (1.7)	0.14 (0.13)	0.14 (0.11)	.071	94 (2)	3.64 (0.02)
15	0.25	149	133 (14)	1.30 (0.39)	19.7 (6.0)	0.07 (0.04)	0 (n=7)	.075	94 (13)	3.67 (0.16)
20	0.25	145	171 (32)	-	24.8 (5.6)	0.01 (0.01)	0 (n=5)	.071	103 (1)	3.94 (0)
25	0.24	139	231 (10)	1.65 (0.25)	32.1 (1.2)	0.06 (0.01)	0 (n=8)	.073	103 (11)	3.81 (0.06)
30	0.24	150	234 (19)	0.85 (0.11)	35.1 (1.2)	0 (n=8)	0 (n=8)	.063	108 (8)	4.34 (0.38)

* Organic-N

** Nitrate-N

NOTE: Numbers in parentheses are standard deviations of measurements. If measured value is zero, the number of replicates is given.

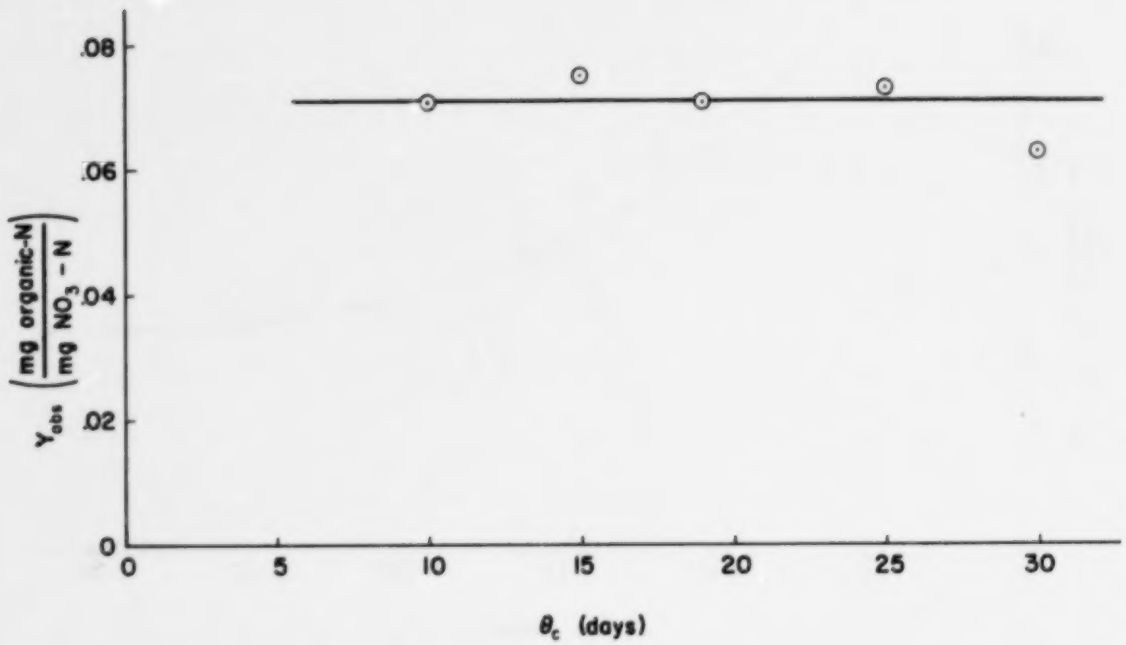


Figure 10. Observed biomass yield versus solids retention time.

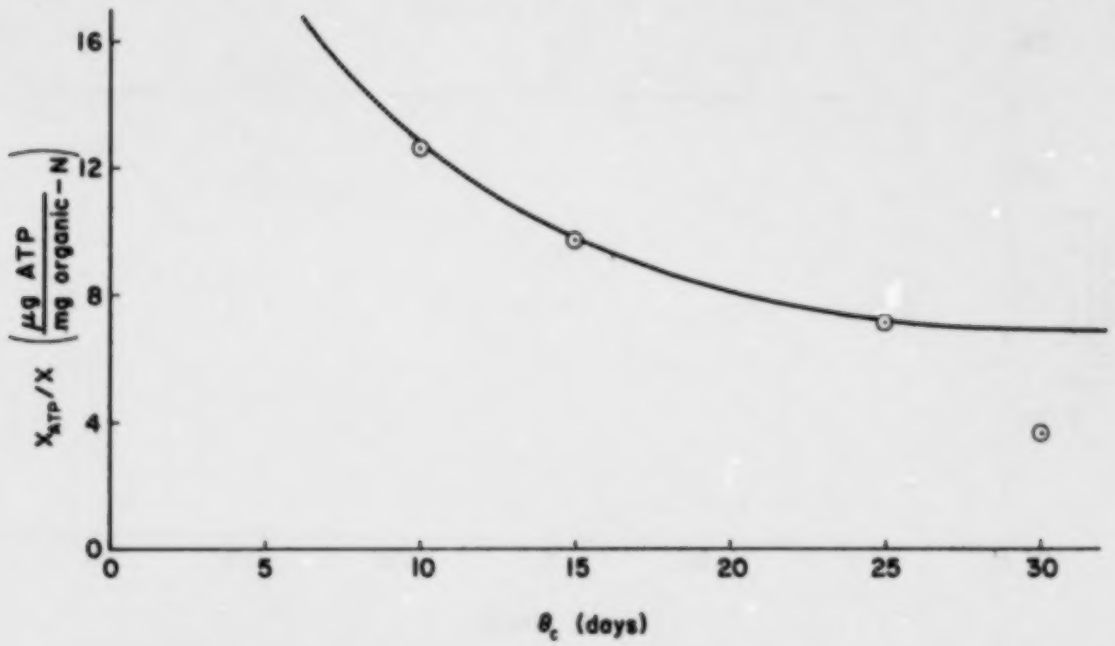


Figure 11. ATF content of biomass versus solids retention time.

effect on reaction kinetics. Solids retention time for each reactor was chosen to enable operation within 10-20 percent of the estimated minimum attainable θ_c for that value of S/X. Table 12 presents a summary of results from these experiments along with results for the reactor operated at S/X = 142 mg S/mg organic-N and θ_c = 10 days in the variable growth rate experiments. Values for the maximum attainable unit rate of nitrate removal ($U_{m,a}$) were calculated from results of transient rate tests using a least squares regression technique (Equations 32-35). Data from these tests are presented in Tables B3-B6 in the Appendix. Figure 12 graphically illustrates the dependence of $U_{m,a}$ on S/X. The linear least squares regression line calculated to describe this relationship is:

$$U_{m,a} = 0.19 + 0.01\ (S/X) \qquad (37)$$

$U_{m,a}$ = maximum attainable unit rate of nitrate removal, $\left[\frac{\text{mg } NO_3\text{-N}}{\text{mg organic-N-day}}\right]$;

S/X = ratio of sulfur to biomass concentration in reactor, [mg S/mg organic-N].

Filtered (0.45 μm pore size) samples from the reactors operated at S/X = 45, 56, 100, 194 mg S/mg organic-N were analyzed for COD and an average value of 10 mg/ℓ was obtained.

Effect of temperature--Continuous feed reactors were also operated at 12°C and 30°C to evaluate the temperature dependence of $U_{m,a}$. These reactors were operated at approximately the same S/X and, at solids retention times near the estimated minimum attainable value for each temperature. Table 13 presents the results of these experiments plus the experiment conducted at 21°C and 10-day θ_c during the variable growth rate experiments. Results obtained during the transient rate tests used to determine $U_{m,a}$ are presented in the Appendix in Tables B7 and B8. Results of these transient tests indicate that nitrite accumulation was much more pronounced at 30°C than at 12°C. Figure 13 is an Arrhenius plot used to determine the apparent activation energy for $U_{m,a}$ (Equation 7). A least squares regression on the linearized data yielded an activation energy of 13.2 kcal/mole.

Settling and thickening characteristics--Data from zone settling tests on slurries taken from continuous reactors operated at three different S/X values are presented in Appendix Tables B9, B10, and B11. Figure 14 shows the solids fluxes calculated from these data as functions of X_t.

Four flocculent settling tests were performed on three different continuous culture slurries. Two tests with different initial solids concentration were performed on the slurry with S/X equal to 150. Appendix Tables B12 through B15 present the data from these tests. The results were analyzed using isoconcentration plots on depth vs time graphs such as the one presented in Figure 15. Figure 16 shows the relationship between effluent suspended solids and overflow rate for these slurries. Table 14 shows the regression

TABLE 12. SUMMARY OF CONTINUOUS CULTURE RESULTS AT VARIOUS S/X

Kinetic parameters		Operating parameters		Mixed liquor characteristics		Effluent characteristics		Stoichiometric parameters		
S/X ($\frac{mg\ S}{mg\ N^*}$)	$U_{m,a}$ ($\frac{mg\ N^{**}}{mg\ N^*\text{-Day}}$)	θ_c (d)	θ (d)	S ($\frac{g\ S}{\ell}$)	X ($\frac{mg\ N^*}{\ell}$)	NO_3^--N ($\frac{mg}{\ell}$)	NO_2^--N ($\frac{mg}{\ell}$)	Y_{obs} ($\frac{mg\ N^*}{mg\ N^{**}}$)	ΔSO_4-S ($\frac{mg\ S}{\ell}$)	ΔAlk ($\frac{meq}{\ell}$)
194	2.13	8	0.26	17.33 (0.75)	90 (8)	0 (n=5)	0 (n=5)	.095	96 (8)	3.80 (0.14)
142	1.62	10	0.25	11.80 (1.70)	83 (10)	0.14 (0.13)	0.14 (0.11)	.071	94 (2)	3.64 (0.02)
100	1.22	13	0.24	15.20 (1.96)	152 (12)	0.02 (0.02)	0.003 (0.003)	.094	93 (6)	3.54 (0.26)
56	0.74	20	0.25	11.30 (0.50)	203 (7)	0.01 (0.004)	0 (n=5)	.084	100 (9)	3.81 (0.12)
45	0.64	30	0.24	12.27 (0.24)	275 (14)	0.04 (0.02)	0.001 (0.0004)	.075	93 (12)	3.33 (0.12)

* Organic-N

** Nitrate-N

NOTE: Numbers in parentheses are standard deviations of measurements. If the measured value is zero, the number of replicates is given.

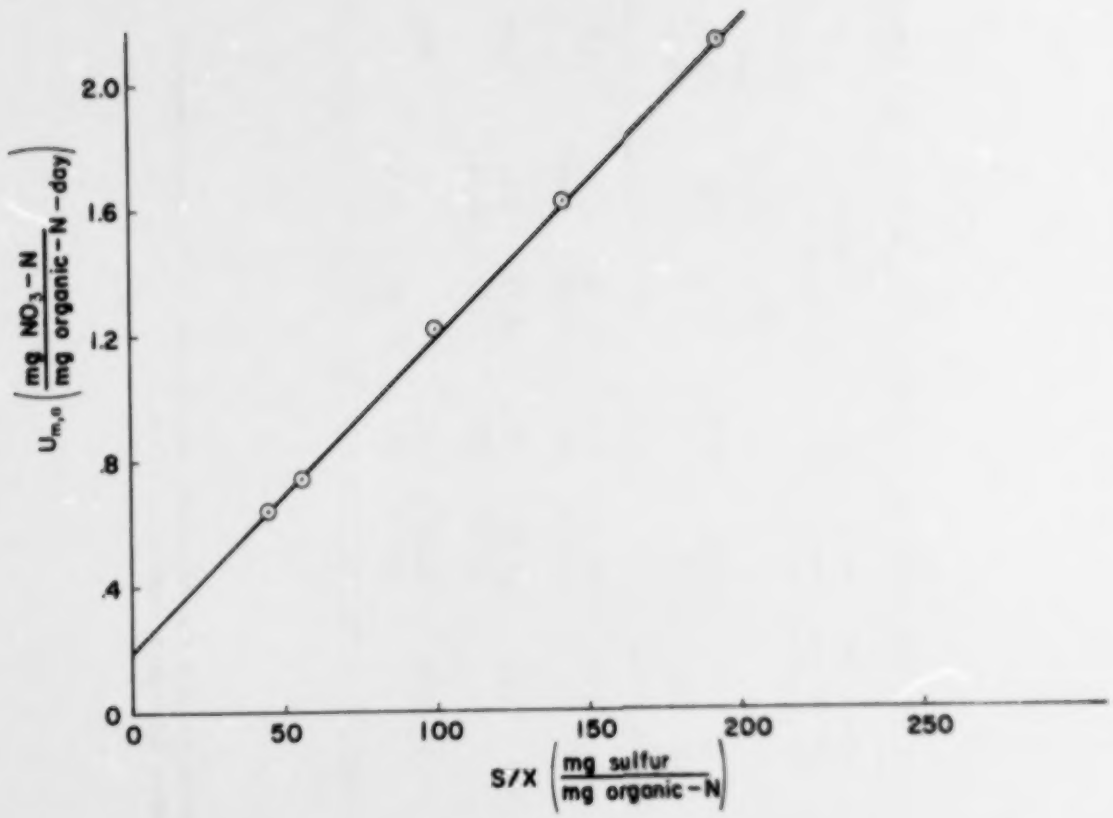


Figure 12. Maximum attainable unit rate of denitrification versus sulfur to biomass ratio.

TABLE 13. SUMMARY OF CONTINUOUS CULTURE RESULTS AT VARIOUS TEMPERATURES

Kinetic parameters			Operating parameters		Mixed liquor characteristics		Effluent characteristics		Stoichiometric parameters		
T (^{o}C)	$U_{m,a}$ ($\frac{mg\ N}{mg\ N^{*}\text{-day}}$)	S/X ($\frac{mg\ S}{mg\ N^{*}}$)	θ_c (d)	θ (d)	S ($\frac{g\ S}{\ell}$)	X ($\frac{mg\ N^{*}}{\ell}$)	NO_3^--N ($\frac{mg}{\ell}$)	NO_2^--N ($\frac{mg}{\ell}$)	Y_{obs} ($\frac{mg\ N^{*}}{mg\ N^{**}}$)	ΔSO_4-S ($\frac{mg\ S}{\ell}$)	ΔAlk ($\frac{meq}{\ell}$)
12	0.97	144	15	0.26	25.14 (1.15)	175 (10)	0.01 (0.01)	0 (n=6)	.100	92 (2)	3.87 (0.11)
21	1.62	142	10	0.25	11.80 (1.70)	81 (10)	0.14 (0.13)	0.14 (0.11)	.071	94 (2)	3.64 (0.02)
30	3.92	141	7.6	0.24	10.57 (0.53)	75 (8)	0 (n=6)	0 (n=6)	.080	88 (6)	3.16 (0.21)

* Organic-N

** Nitrate-N

NOTE: Numbers in parentheses are the standard deviations of the measurements. For values equal to zero, the number of measurements is given.

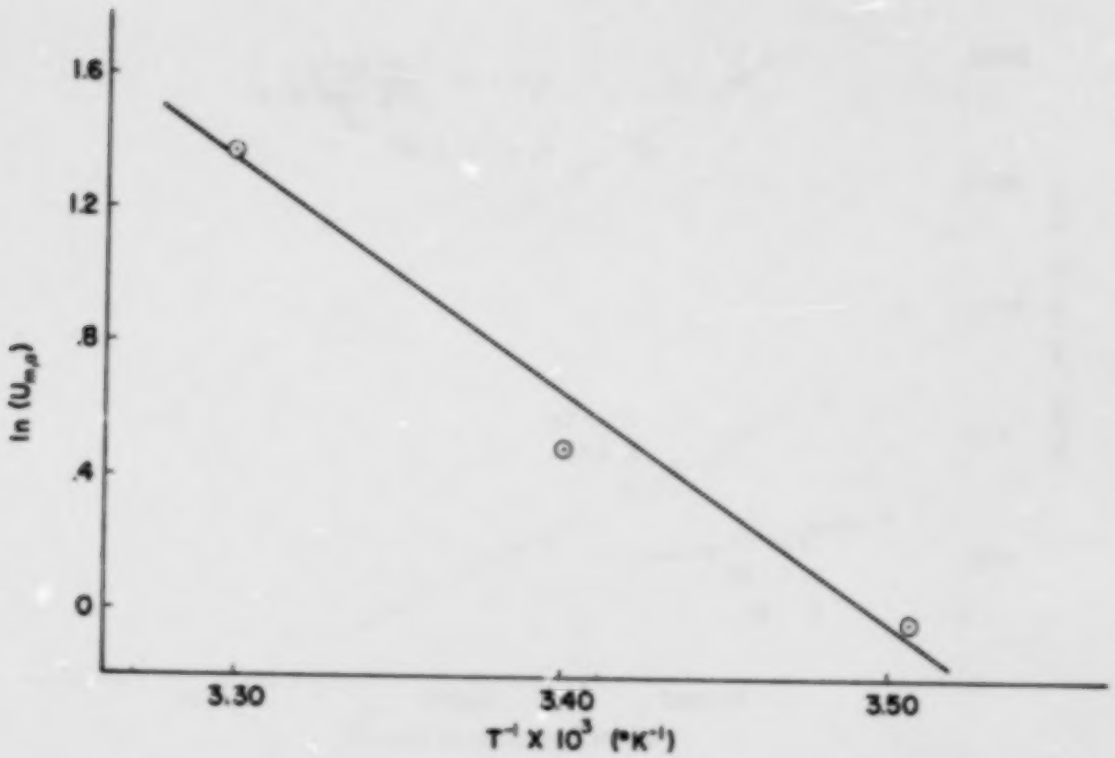


Figure 13. Natural logarithm of maximum attainable unit rate of denitrification versus inverse of absolute temperature.

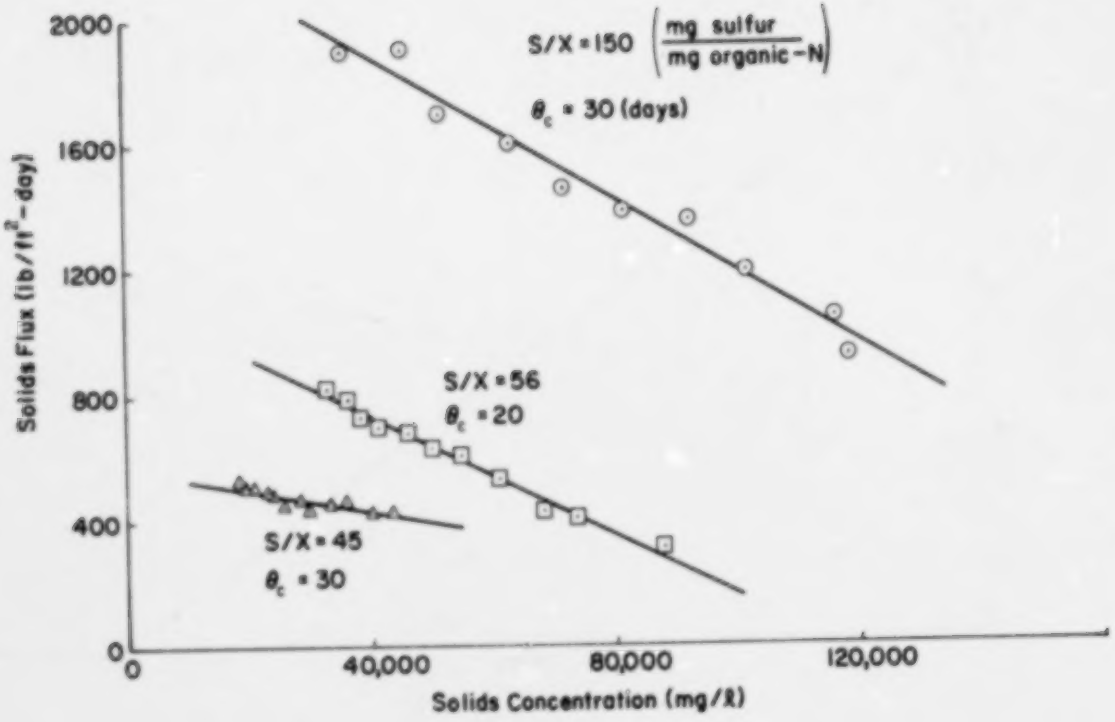


Figure 14. Solids flux versus solids concentration for various values of the sulfur to biomass ratio.

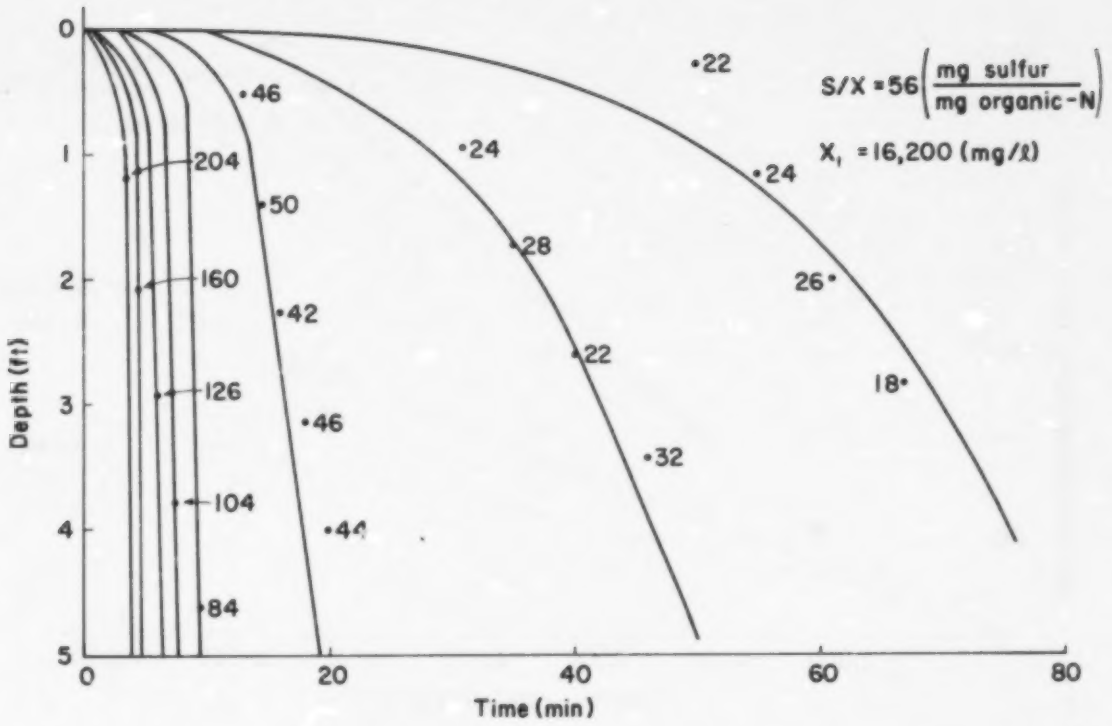


Figure 15. Suspended solids concentration on depth versus time graph with lines of isoconcentration.

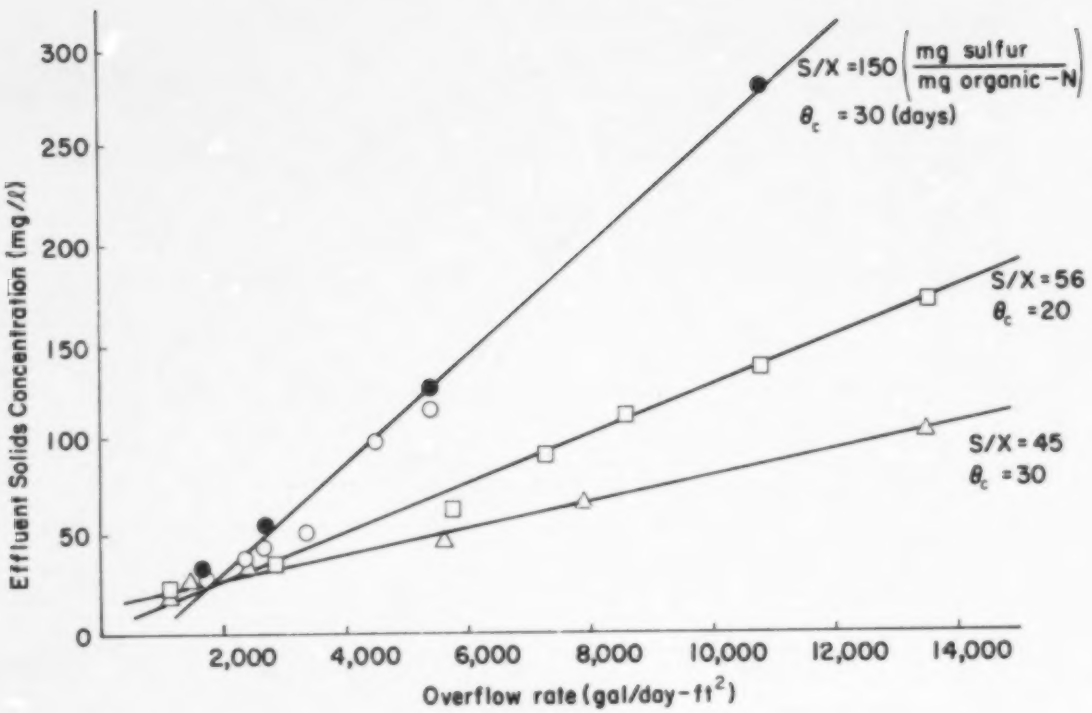


Figure 16. Effluent suspended solids concentration versus overflow rate for various values of the sulfur to biomass ratio.

TABLE 14. REGRESSION EQUATIONS FOR RESULTS OF SOLIDS SEPARATION TESTS

Solids flux regression equations

S/X	Equation
45	$G_s = 568 - (3.49 \times 10^{-3})X_t$
56	$G_s = 1110 - (9.44 \times 10^{-3})X_t$
150	$G_s = 2330 - (1.13 \times 10^{-3})X_t$

G_s = solids flux due to subsidence, [lb/ft^2-day];

X_t = total solids concentration, [mg/ℓ].

Effluent solids regression equations

S/X	
45	$X_t^{eff} = 14.8 + (6.34 \times 10^{-3})\frac{Q}{A_c}$
56	$X_t^{eff} = 2.2 + (1.21 \times 10^{-2})\frac{Q}{A_c}$
150	$X_t^{eff} = -23.6 + (2.70 \times 10^{-2})\frac{Q}{A_c}$

X_t^{eff} = total solids concentration in effluent, [mg/ℓ];

$\frac{Q}{A_c}$ = overflow rate, [gpd/ft^2].

equations derived from results of solids separation tests.

Discussion of Phase I Experimental Results

Observed Yield Coefficients--

A comparison of values of the observed biomass yield (Y_{obs}) in the Phase I continuous culture experiments (Tables 11, 12, and 13) indicates only minor variation of this parameter with growth rate ($\frac{1}{\theta_c}$) or S/X.

Table 15 presents average values of stoichiometric coefficients measured in the Phase I batch and continuous culture experiments. Table 15 also shows predicted values of the coefficients derived from a balanced stoichiometric equation incorporating the cell formula $C_5H_7O_2N$, and the measured average biomass yield. There is little difference between the two reactor systems in observed biomass yields, but there is a large difference in the coefficient for sulfate production ($Y^{SO_4\text{-}S}_{obs}$). This is probably due to oxygen leaks in the continuous reactors which allowed excess sulfur to be microbially oxidized. The continuous reactors were initially adjusted to keep oxygen leaks, as measured on an electron equivalent basis, to less than 10 percent of the equivalent nitrate concentration in the feed. During reactor operation, however, oxygen leaks increased as indicated by increased alkalinity destruction. An oxygen leak of approximately 30 mg O_2/hr was estimated by assuming that alkalinity destruction and sulfate production occurred with the same stoichiometry as measured in the batch reactors. This is equivalent to an increase in the influent nitrate concentration of 11 mg/ℓ NO_3^--N. If a leak of this magnitude actually were to occur, the observed yield would be calculated as 0.060 mg organic-N/mg NO_3^--N by equating oxygen and nitrate electron equivalents.

Balanced Stoichiometric Equation--

An empirical cell mass formula of $C_5H_7O_2N$ is often used in applying stoichiometric principles to microbial reactions (33,38,41,47). To confirm the validity of this formula for autotrophic denitrification, stoichiometric coefficients $Y^{SO_4\text{-}S}_{obs}$ and Y^{Alk}_{obs} were calculated using measured values of Y_{obs} from Phase I and a cell synthesis equation incorporating the assumed protoplasm formula. The values shown in Table 15 indicate that $Y^{SO_4\text{-}S}_{obs}$ and Y^{Alk}_{obs} are in good agreement with the corresponding predicted values for batch experiments. However, observed and predicted values of these parameters do not agree for continuous cultures. The higher values of $Y^{SO_4\text{-}S}_{obs}$ and Y^{Alk}_{obs} are probably a result of the oxygen leaks in the continuous reactors. However, the observed ratio of these parameters ($Y^{SO_4\text{-}S}_{obs}/Y^{Alk}_{obs}$) agrees well with the predicted value, indicating that the use of the cell formula $C_5H_7O_2N$ is reasonable for autotrophic denitrification.

The suggested method for calculating reaction stoichiometry for autotrophic denitrification is to use the average value of Y_{obs}, 0.08 mg organic-N/mg NO_3^--N, along with a cell formula of $C_5H_7O_2N$ to produce the following balanced stoichiometric equation.

$$1.0\ NO_3^- + 1.10\ S + 0.40\ CO_2 + 0.76\ H_2O + 0.080\ NH_4^+ \rightarrow 0.080\ C_5H_7O_2N + 0.50\ N_2 + 1.10\ SO_4^= + 1.28\ H^+ \quad (38)$$

TABLE 15. STOICHIOMETRIC COEFFICIENTS FOR BATCH AND CONTINUOUS CULTURES

	Y_{obs} $(\frac{\text{mg organic-N}}{\text{mg } NO_3^- \text{-N}})$	$Y_{obs}^{SO_4\text{-S}}$ $(\frac{\text{mg } SO_4\text{-S}}{\text{mg } NO_3^- \text{-N}})$	Y_{obs}^{Alk} $(\frac{\text{meq}}{\text{mg } NO_3^- \text{-N}})$	$Y_{obs}^{SO_4\text{-S}} / Y_{obs}^{Alk}$ $(\frac{\text{mg } SO_4\text{-S}}{\text{meq}})$
Continuous, average	0.08	3.2	0.12	26
Batch, average	0.08	2.4	0.11	21
Continuous, predicted	----	2.5	0.09	28
Batch, predicted	----	2.4	0.09	25

To use this equation for predicting changes in composition of a given wastewater during treatment, the equivalent nitrate-nitrogen concentration in the feed must be calculated. The definition of equivalent nitrate-nitrogen concentration (CEN) presented in Equation 32 can be extended to include dissolved oxygen--another biologically available electron acceptor. Calculation of CEN is based on the assumption that the stoichiometry, expressed on an electron equivalent basis, is the same for any biologically available oxidant. This is probably not strictly correct, since the free energy available from different oxidations is not the same. However, this assumption will not cause major errors, since in most wastewaters to be denitrified the concentrations of oxygen and nitrite will be small, compared to nitrate on an electron equivalent basis. Therefore, the equivalent nitrate-nitrogen concentration may be computed by the following formula.

$$CEN = N + 0.6\ NI + 0.35\ D.O. \tag{39}$$

D.O. = dissolved oxygen concentration, [mg/ℓ].

The amount of sulfate-sulfur produced (ΔSO_4-S) and the amount of alkalinity destroyed (ΔAlk) during autotrophic denitrification can be calculated as a function of the amount of equivalent nitrate-nitrogen removed (CEN^o-CEN). The stoichiometric coefficients calculated from Equation 38 and presented in Table 15 are used in the calculation.

$$\Delta SO_4\text{-}S = Y_{obs}^{SO_4\text{-}S} \ (CEN^o\text{-}CEN) \tag{40}$$

$$\Delta Alk = Y_{obs}^{Alk} \ (CEN^o\text{-}CEN) \tag{41}$$

Sulfur Balance--

A sulfur balance was performed on the Phase I continuous reactor operated at S/X = 100 to determine if there were major sulfur oxidation products besides sulfate. Sulfide odors were never detected in the effluent but other sulfur by-products could have been present. The material balance on sulfur in a slurry reactor can be used to predict reactor sulfur concentrations.

$$S = \theta_c \left[\frac{cN^o}{\theta} - \frac{\Delta SO_4\text{-}S}{\theta} - \frac{S^{eff}}{\theta}\right] \tag{42}$$

The amount of sulfur added to the reactor each day was calculated according to the desired feed ratio and the flow since the last feeding. This amount varied somewhat, so a weighted average daily feed rate was calculated. The proper weighting function was determined by consideration of the concept of residence time distribution (RTD) (28,29). This function gives the fraction of particles which entered the reactor together, that have left the reactor at any given time. The function, 1-RTD, will give the fraction still within the reactor. This function will weight the variable sulfur feed rates to best estimate reactor sulfur concentration. The RTD for the solids in a completely mixed reactor with recycle can be obtained by substituting θ_c for θ in the RTD for a completely mixed reactor without recycle.

$$1 - RTD = e^{-t/\theta_c} \tag{43}$$

Effluent elemental sulfur and sulfate concentration were measured at several times before the steady state reactor sulfur concentration was measured. The average values for S^{eff} and ΔSO_4-S were 11 mg/ℓ S and 104 mg/ℓ S, respectively. The predicted value of S was 14,900 mg/ℓ S, which compared very well with the average measured value of 15,200 mg/ℓ S.

Composition of Reactor Gas--

No nitric oxide and only a trace of nitrous oxide (50 ppm) was detected in the Phase I continuous reactor recirculating gas. A trace of nitrous oxide was also detected in the nitrogen gas added to the reactors, so the relative amounts of nitrogenous gases produced during autotrophic denitrification cannot be firmly established. Absence of significant amounts of nitrogenous oxides, however, indicates that elemental nitrogen is the primary end-product of nitrate reduction. This is consistent with previous analyses which report no production of nitrous or nitric oxide during heterotrophic denitrification (48-51). No gas analyses were performed in any of the other experimental phases.

Kinetics--

Results of Phase I continuous culture experiments can be used to calculate kinetic coefficients. The average steady state effluent nitrate-nitrogen concentration measured during these experiments was 0.03 mg/ℓ NO_3^--N. An

exact value for the saturation constant in NFN (K_n) could not be calculated from these measurements because they were too low to be accurately analyzed. However, this value (0.03 mg/ℓ) can be used as a conservative estimate of K_n since most reactors were operated near the maximum attainable unit rate. Values of K_n measured for heterotrophic denitrification (0.06 (52), 0.08 (53), and 0.16 (54) mg/ℓ NO_3^--N) are of the same order of magnitude. Because the estimated value of K_n is so small, effluent nitrate concentrations from steady state autotrophic denitrification slurry reactors will be negligible for all feasible operating conditions. Any steady state reactor which is operated such that $\theta_c > \theta_c^{m,a}$ should produce an effluent with negligible nitrate nitrogen.

Equation 4 represents the dependence of $U_{m,a}$ on the ratio S/X as a saturation function. The exact form of this function is such that at small values of S/X there is an extended region in which $U_{m,a}$ is proportional to S/X (Figure 2). The continuous culture experiments with variable S/X were designed to operate in this region, since the process requirement for elemental sulfur is reduced at lower values of S/X. Therefore, values of $U_{m,a}$ measured in the variable S/X experiments would be expected to be linearly related to S/X with a zero intercept.

The results of the variable S/X experiments presented in Figure 12 show an excellent linear relationship between $U_{m,a}$ and S/X with a small, but not insignificant, non-zero intercept. This behavior could be due to variations in sulfur particle size among the various experiments, or to uneven distribution of biomass over sulfur surface area.

The effect of variation in sulfur particle size would be expected in the series of variable S/X experiments because those reactors were operated at different values of θ_c. Those reactors operated at higher values of θ_c would retain the sulfur particles for a longer time, resulting in increased microbial oxidation and reduced size. Smaller sulfur particles would present more surface area for microbial growth per unit sulfur mass. Therefore, the variable which exerts the primary influence on the unit rate of denitrification--the sulfur surface area--would be underestimated by measurement of sulfur mass in reactors operated at high values of θ_c. This error in measurement would tend to shift points at low values of S/X (high θ_c) to the right in Figure 12 and move the intercept closer to the origin.

The effect of an uneven distribution of biomass could be explained using the concept of an "effective sulfur" concentration (S_e). Such a variable would represent the concentration of sulfur actually covered with biomass. The kinetic model for autotrophic denitrification assumes that biomass in the reactor is distributed evenly over the available sulfur surfaces. This would result in a constant biofilm thickness on every sulfur particle. In practice, however, it is probable that as the amount of sulfur relative to biomass increases a larger fraction of sulfur will not be incorporated into a biofilm matrix. At high values of S/X there will be relatively less microbial "glue" available to capture sulfur particles. Experimental evidence of this behavior was observed in the flocculent settling tests, where more

suspended solids were found at higher values of S/X (Figure 1f). Thus, a kinetic model might also be developed using S_e/X rather than S/X as its primary variable. If these latter assumptions were applied to the experimental results, one could expect that the slope of the regression line relating $U_{m,a}$ to S/X would increase and its intercept would approach zero.

The kinetics of autotrophic denitrification at high values of S/X were investigated in semicontinuous reactors operated with different sulfur to nitrate-nitrogen feed ratios (c). Unfortunately, the experiments were conducted early in the study, before the importance of S/X as the primary kinetic variable was recognized. However, it is possible to relate the sulfur to nitrate-nitrogen feed ratio to S/X. The material balance equation showing the relationship between S/X and c is shown in Table 1. Inspection of this equation shows that c will be proportional to S/X when c is significantly greater than the stoichiometric ratio (ν); effluent nitrate-nitrogen concentration (N) is low; and, the observed biomass yield (Y_{obs}) is constant. Since these conditions were generally met during the culture characterization experiments, c can be used as a surrogate measure of S/X. Since the nitrate feed rate to each reactor was the same, and since the biomass yield should be constant, biomass concentrations in the reactors should have been equal. Therefore, gas production rate which was used to represent the rate of denitrification could also be used as a surrogate measure of $U_{m,a}$.

The results presented in Figure 7 support the prediction of the model for the behavior of $U_{m,a}$ at high values of S/X, when considered in terms of the surrogate variables. The results show that in general the relationship between gas production rate (surrogate for $U_{m,a}$) and c (surrogate for S/X) is that of a saturation function. In particular, the hyperbolic tangent function drawn in Figure 7 to represent the relationship is in good agreement with the experimental results.

Temperature Effects--

Possible effects of diffusional limitations on the observed temperature dependence of autotrophic denitrification should be considered when comparing these results with information from other denitrification or sulfur oxidation systems. The kinetic model developed for autotrophic denitrification predicts that the observed rate of denitrification will be limited by transport processes whenever the rate is a linear function of S/X. The range of S/X values used in these experiments resulted in a linear relationship between $U_{m,a}$ and S/X (Figure 12), so the rates measured in this study were probably limited by intra-film transfer of sulfur. Rates of heterogeneous chemical reactions measured under conditions of diffusion limitations are known to exhibit apparent activation energies equal to half the true value (28,29). Therefore, the activation energy for the actual microbial reaction in autotrophic denitrification should be approximately twice the measured value, i.e., 26 kcal/mole. Results of temperature dependence studies on the aerobic oxidation of sulfur by Thiobacillus thiooxidans under conditions of an excess of sulfur (55) can be used to calculate an activation energy of 23.6 kcal/mole. If the temperature dependence displayed by this organism is similar to that of Thiobacillus denitrificans, then the model's conclusion that observed

rates of denitrification are limited by intra-film transport of sulfur appears valid.

Settling and Thickening--

Several techniques for data analysis have been proposed for use with the batch flux method to describe the solids separation process (30,31,56). Results of zone settling tests performed on sulfur-biomass slurries indicate that a linear function best describes the relationship between solids flux and solids concentration (Figure 14). These results could also have been analyzed according to the more frequently used logarithmic or semi-logarithmic relationships. However, the linear model was used because it resulted in a somewhat better fit to the data over the range of experimental observations.

Applying the batch flux analysis technique to a slurry with a linear solids flux leads to the conclusion that there are no limitations on the solids separation process due to thickening (57). The process is limited only by clarification, i.e., the ability of the slurry entering the clarifier-thickener to settle at a rate faster than the overflow rate (Q/A_c). The maximum allowable overflow rate set by clarification limitations can be calculated from results of zone settling tests according to the following equation (57):

$$(Q/A_c)^{max} = \frac{M(X_t^r - X_t^a)^2}{X_t^r \quad X_t^a} \qquad (44)$$

$(Q/A_c)^{max}$ = maximum allowable overflow rate, $[m^3/m^2\text{-}d]$;

M = slope of solids flux vs total solids concentration curve, [m/d];

X_t^r = total solids concentration in recycle line, [mg/ℓ];

X_t^a = total solids concentration in reactor, [mg/ℓ].

Although zone settling tests predict limits on the operation of solids separation processes, they do not predict effluent quality. Results of flocculent settling tests predict the relationship between the operating variable for solids separation (Q/A_c) and effluent quality (X_t^{eff}) (Figure 16). These results indicate improved settling properties at lower values of S/X. For a given overflow rate, predicted effluent suspended solids concentration decreases as S/X decreases. This is probably due to the fact that as S/X decreases the amount of biomass relative to sulfur increases. This increases the chance that a sulfur particle will become enmeshed in a biomass matrix and be removed with the larger agglomerates. This observation is consistent with the postulate of an effective sulfur concentration used previously to explain kinetic behavior.

Initial solids concentration seemed to have relatively minor effect on the flocculent settling characteristics of sulfur-biomass slurries. Two initial solids concentrations were used to obtain settling data for the

slurry with S/X = 150 mg S/mg organic-N. The open and filled circles in Figure 16 represent data obtained at initial solids concentrations of 67,000 mg/ℓ and 35,600 mg/ℓ , respectively. These results were calculated using a depth of five feet. There is very little indication that separation efficiency depends on depth, as indicated by the nearly vertical isoconcentration lines in Figure 15.

PHASE II - THIOSULFATE AND SULFIDE EXPERIMENTS

A series of completely mixed semi-continuous flow reactors were employed in Phase II, to determine whether autotrophic denitrification with T. denitrificans using sulfide or thiosulfate as an electron source was feasible. This experimental phase was carried out in four experiments, as described below. Table 16 summarizes the experiments performed under Phase II.

TABLE 16. EXPERIMENTAL PROGRAM - PHASE II (STEADY-STATE)

Experiment number	θ_c (days)	C_f	Feed sulfur form
1	15	17.7	$S_2O_3^=$
	10	17.7	$S_2O_3^=$
	5	17.7	$S_2O_3^=$
2	15	4.1	$S_2O_3^=$
	10	4.1	$S_2O_3^=$
	5	4.1	$S_2O_3^=$
3	10	2.0	$S_2O_3^=$
	10	0.8	$S_2O_3^=$
	10	0.5	$S_2O_3^=$
4	10	1.7	$S^=$

Experiment #1-Effect of Growth Rate - High C_F

Experimental Plan and Techniques--

The purpose of Experiment #1 was to determine the effect of growth rate on denitrification in systems which were fed high feed ratios, C_F of thiosulfate. The basic feed solution components used in this experiment are given in Table 17. To these basic ingredients $Na_2S_2O_3 \cdot 5H_2O$ was added to

TABLE 17. PHASE II - FEED SOLUTION NUTRIENTS

Constituents	Concentration (milligrams/liter)
KNO_3	25 (as N)
$MgCl_2 \cdot 6H_2O$	500
$FeSO_4$	10
NH_4Cl	25
KH_2PO_4	300
K_2HPO_4	1,500
$NaHCO_3$	1,000
Tap water	to one liter volume

make up the appropriate feed ratio. The pH of the feed and the reactor was maintained at about 7. The reactor in this experiment was seeded from enriched cultures of Thiobacillus denitrificans grown on elemental sulfur in Phase I.

The reactor was operated in a completely mixed, semi-continuous flow mode. Biomass solids wasting and feeding was done once per day such as to control the microorganism net specific growth rate ($1/\theta_c$) at levels indicated in Table 16. A gas collection system was used to indicate denitrification activity and to keep the systems anaerobic. Figure 17 is a schematic of the reactor. The reactor volume was four liters.

The reactor was run until steady-state conditions were reached, then steady-state data was collected for a seven-day period. Reactor sampling was done daily for thiosulfate, nitrite, nitrate, pH and volatile suspended solids.

Experimental Results--

The steady state experimental results of Experiment #1 - Phase II are summarized in Table 18. The values shown in this table are the mean of seven days of steady-state data.

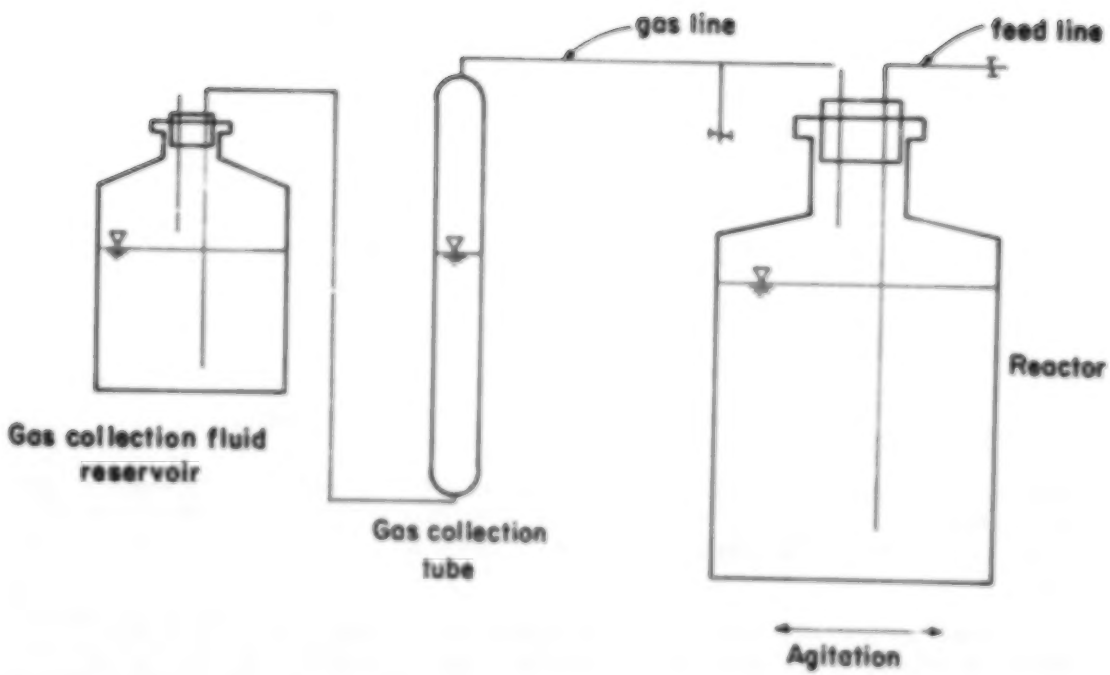


Figure 17. Semicontinuous flow sulfide/thiosulfate reactor.

TABLE 18. SUMMARY OF STEADY-STATE EXPERIMENTAL RESULTS - PHASE II

Expt. No.	θ_c (d)	Avg. feed data (mg/l) NO_3^--N	$S_2O_3^=$-S	C_F	Avg. effluent data (mg/l) NO_3^--N	NO_2^--N	$S_2O_3^=$-S	Volatile suspended solids	Y_{obs}	Measured C_R
1	15	25	1246	17.7	<0.2	<0.05	1120	25	1.0	1.8
	10	25	1246	17.7	<0.2	<0.05	1101	34	1.4	2.1
	5	25	1246	17.7	<0.2	<0.05	1126	20	0.8	1.7
2	15	22	258	4.1	<0.2	<0.05	191	12	0.5	1.1
	10	22	258	4.1	<0.2	<0.05	186	9	0.4	1.1
	5	22	258	4.1	<0.2	<0.05	172	24	1.0	1.4
3	10	25	143	2.0	0.6	<0.05	66	27	1.1	1.1
	10	26	55	0.8	1.6	0.08	<2.5	34	1.4	1.3
	10	25	33	0.5	15.0	1.4	<2.5	-	-	1.3
	10	100	243*	1.7	21.7	0.43	0.8*	122	1.6	2.2

* Sulfide

Experiment #2 - Effect of Growth Rate - Intermediate C_F

Experimental Plan and Techniques--

Experiment #2 was designed to determine the effect of θ_c on denitrification in systems with intermediate feed ratios of thiosulfate. Reactor operation was the same as for Experiment #1 except that the feed ratio was lowered to 4.1.

Experimental Results--

The steady-state results of Experiment #2 - Phase II are summarized in Table 18. The values shown in this table are the mean of seven days of steady-state data.

Experiment #3 - Determination of Consumptive Ratio

Experimental Plan and Techniques--

The purpose of Experiment #3 was to determine the consumptive ratio, C_R, and to observe system operation under thiosulfate limiting growth conditions. After the steady-state data for this experiment was collected, the reactor was used to perform a dynamic study to assess the stability of a thiosulfate system under fluctuating loads (varying C_F values). In this experiment a reactor with thiosulfate limiting growth (C_F = 0.45) was rapidly changed to a nitrate limiting growth reactor (C_F = 3.5) and then rapidly back to a thiosulfate limiting growth system (C_F = 0.45). Of particular concern here was the effect of nitrite buildup (at low C_F values) on the responsiveness of the denitrification process.

Reactor operation was as described in Experiments #1 and #2, except that the feed ratio was maintained at the values indicated in Table 16 for the steady-state study and was allowed to vary, as described above, for the dynamic study.

Experimental Results--

The steady-state results of Expeirment #3 are summarized in Table 18. The values shown in this table are the mean of seven days steady-state data. The results of the dynamic study are presented in Figure 18.

Experiment #4 - Sulfide Experiments

Experimental Plan and Techniques--

Experiment #4 was a feasibility study to determine if sulfide could be used effectively as an electron donor in autotrophic denitrification. This expeirment was conducted in semicontinuous reactors, and operated in a manner similar to Experiment #1, #2 and #3, with the following modifications.

The reactor volume was reduced to one liter, and $Na_2S \cdot 9H_2O$ was added to the basic feed solution listed in Table 17. The feed ratio was adjusted to 1.7. Also, the concentration of phosphate buffer was increased by a factor of ten to compensate for the caustic nature of Na_2S.

Experimental Results--

The results of Experiment #4 are summarized in Table 18. The values

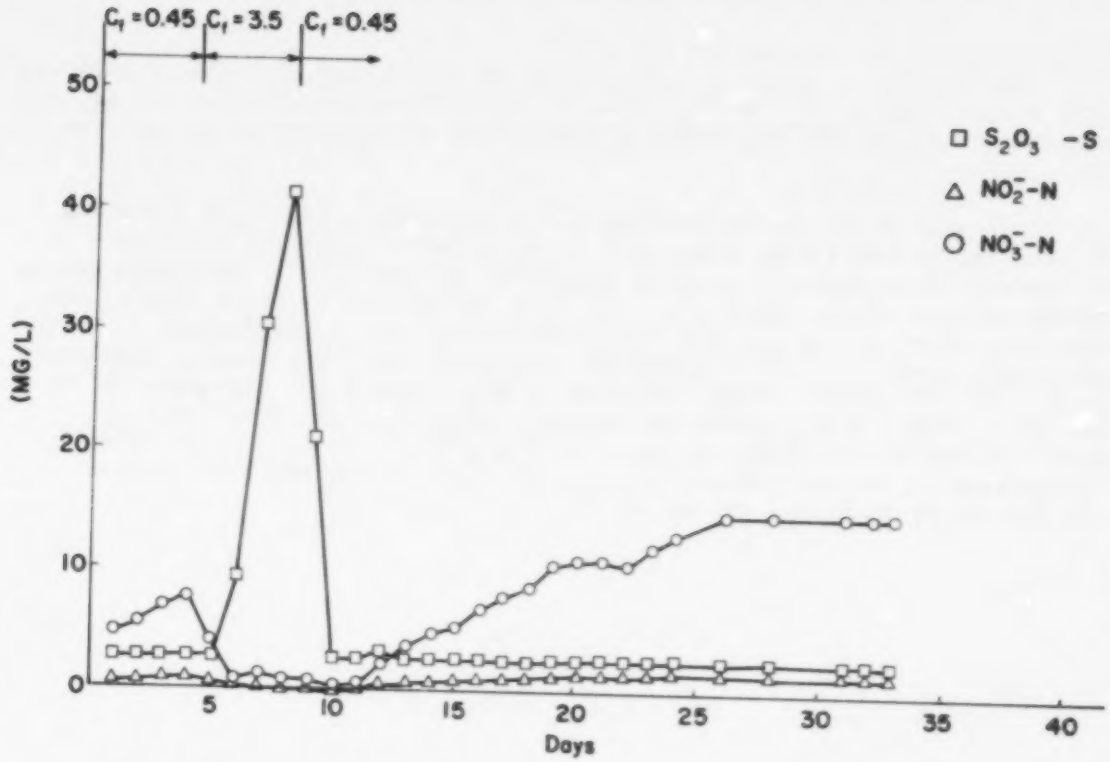


Figure 18. Response of autotrophic denitrifying system to rapid changes in feed ratio.

shown in this table are the mean of seven days steady-state data.

Discussion of Phase II Experimental Results

Thiosulfate and Sulfide as Electron Donors--

The results of Experiments #1 through #4 indicate that either thiosulfate or sulfide can be used effectively as electron sources for autotrophic denitrification. Essentially complete denitrification can be accomplished provided the feed ratio is greater than some minimum value (probably the consumptive ratio). In the thiosulfate system, denitrification was relatively stable with respect to rapidly fluctuating feed ratios.

Observed Yield and Consumptive Ratio--

The results of Phase II experiments, as summarized in Table 18 indicate high variability in Y_{obs} and C_R values. Some of this variability can be attributed to the inherent chemical instability of thiosulfate and sulfide in solution.

Consider first the thiosulfate ion in solution. The thiosulfate ion is composed of two sulfur atoms each of which has a different electronic structure. The thiosulfate ion is formed by the addition of elemental sulfur to the sulfite anion. Even after reacting the two sulfur atoms (one in the elemental state and one in the sulfite anion) remain distinguishable. Given the proper conditions the thiosulfate anion will decompose back to elemental sulfur and the sulfite anion. One way to make thiosulfate decompose is to add acid. Under acidic conditions weakly dissociated sulfurous acid or bisulfite enhances the decomposition of the thiosulfate because it effectively removes the sulfite ion from the product side of the reaction as illustrated by Equations 45, 46 and 47.

$$S_2O_3^{=} \rightarrow S^o + SO_3^{=} \qquad (45)$$

$$H^+ + S_2O_3^{=} \rightarrow HSO_3^- + S^o \qquad (46)$$

$$2H^+ + S_2O_3^{=} \rightarrow H_2SO_3 + S^o \; . \qquad (47)$$

In an actively denitrifying culture of Thiobacillus denitrificans, the utilization of sulfite by T. denitrificans as an electron donor has the same effect on thiosulfate decomposition as does acid.

Decomposition of thiosulfate causes the calculated Y_{obs} to be erroneously high, because the elemental sulfur that is formed gives positive values in the volatile suspended solids analysis. Elemental sulfur which is retained on the glass fiber filter is volatilized easily at 560°C (elemental sulfur boiling point = 450°C). The inherent inaccuracy of the volatile suspended solids analysis prevents meaningful confirmation of this sulfur interference. It is possible to state only that most of the high observed yield values (greater than the predicted 0.7) were observed in the excess thiosulfate reactors.

It should be noted that Y_{obs} as measured in Phase II is on a volatile suspended solids basis, while the Y_{obs} as reported in Phase I is on a nitrogen content of biomass basis. No cellular (biomass) nitrogen determinations were made in the Phase II studies.

The observed yields determined in Phases I and II agree reasonably well with the calculated theoretical Y_{obs} of 0.084 mg organic-N/mg NO_3^--N (0.683 mg VSS/mg NO_3^--N), 0.087 mg organic-N/mg NO_3^--N (0.704 mg VSS/mg NO_3^--N), 0.086 mg organic-N/mg NO_3^--N (0.703 mg VSS/mg NO_3^--N), for elemental sulfur, sulfide, and thiosulfate, respectively.

The experimental determination of C_R is also hindered by the decomposition of thiosulfate. C_R is supposed to account for only that thiosulfate utilized as an electron donor. But chemical analysis does not differentiate between decomposed thiosulfate and biologically consumed thiosulfate. As a result the "apparent" utilization of thiosulfate by decomposition causes the experimentally determined C_R to be erroneously high. Decomposition of thiosulfate is particularly prevalent when there is an excess of thiosulfate in solution (such as with high C_F values). Results given in Table 18 are in accord with this explanation. Reactors with large excess thiosulfate (C_F = 17.7) have experimentally observed C_R values much higher than the predicted value of 1.35. It is very significant that the two reactors which were thiosulfate limiting (no excess thiosulfate) exhibited consumptive ratios very close to the predicted 1.35 value.

It should be noted that the decomposition of thiosulfate has no effect on the total number of electrons that can be theoretically transferred from a quantity of thiosulfate. The decomposition is a disproportionation or autooxidation reaction which involves no external electron transfer.

PHASE III - PACKED BED REACTOR EXPERIMENTS

Because sulfur and sulfide appear to represent the most cost-effective electron sources on an electron equivalent basis, Phase III studies focused on their use for autotrophic denitrification in packed bed reactors. This phase had several specific objectives. One objective was to investigate the effect of sulfur particle size on the minimum hydraulic retention time required for complete denitrification, where elemental sulfur is used as a packing media. Another objective was to investigate the use of dolomitic limestone as a source of alkalinity in sulfur packed columns. In addition, dolomitic limestone was investigated as a packing media for packed bed columns which were fed sulfide as an electron source. The final objective was to assess the influence of organics in the feed solution on the competition between heterotrophic and autotrophic denitrification in packed bed reactors. Phase III studies were conducted in four experiments as described below.

Experiment #1 - Dolomitic Limestone Reactors - Sulfide Feed

Experimental Plan and Techniques--

In this experiment sulfide was used as an electron donor in columns packed with dolomitic limestone. Two dolomite packed bed reactors were

operated at a hydraulic retention time (θ = bed pore volume/feed flow rate) of approximately 9.25 hours.

These reactors were operated at different feed ratios. In the first reactor (Experiment #1a) the feed ratio was 3.1 and in the second reactor (Experiment #1b) the feed ratio was changed to 0.96 after an extended period of growth under nitrate limiting growth conditions at $C_F \cong 3.1$.

A schematic of the system in Experiments #1a and 1b used is shown in Figure 19. A feed solution containing approximately 25 mg/ℓ NO_3^--N (see Table 19) and the appropriate amount of Na_2S was adjusted to a pH of 9.5 with phosphoric acid and placed in the feed tank. This feed was pumped to a mixing chamber where the pH was adjusted to 7.8 with a pH controller unit.

TABLE 19. PHASE III FEED CHARACTERISTICS (EXPTS. #1, #2 & #3)

Expt. no.	#1	#2	#3
	concentration (mg/ℓ)		
KNO_3-N	25	50	50
$MgCl_2 \cdot 6H_2O$	-	1	1
$FeSO_4 \cdot 7H_2O$	-	1	1
$CaCl_2 \cdot 2H_2O$	-	1	1
$MnCl_2 \cdot 4H_2O$	-	1	1
H_3PO_4	adjust pH of stock solution to 9.5	-	-
K_2HPO_4	-	10	10
$NaHCO_3$	-	1000	variable
Tap water	to volume	to volume	to volume

The feed solution was initially adjusted to pH 9.5 to prevent loss of H_2S from the feed solution. The feed was then passed upward through a dolomitic limestone media reactor (dolomite size range was 3 to 13 mm). Dolomite was selected as the packing because it provided a relatively cheap source of alkalinity while serving as the reactor support media.

To decrease start-up time enriched cultures of Thiobacillus denitrificans were developed on a thiosulfate feed solution and then introduced into

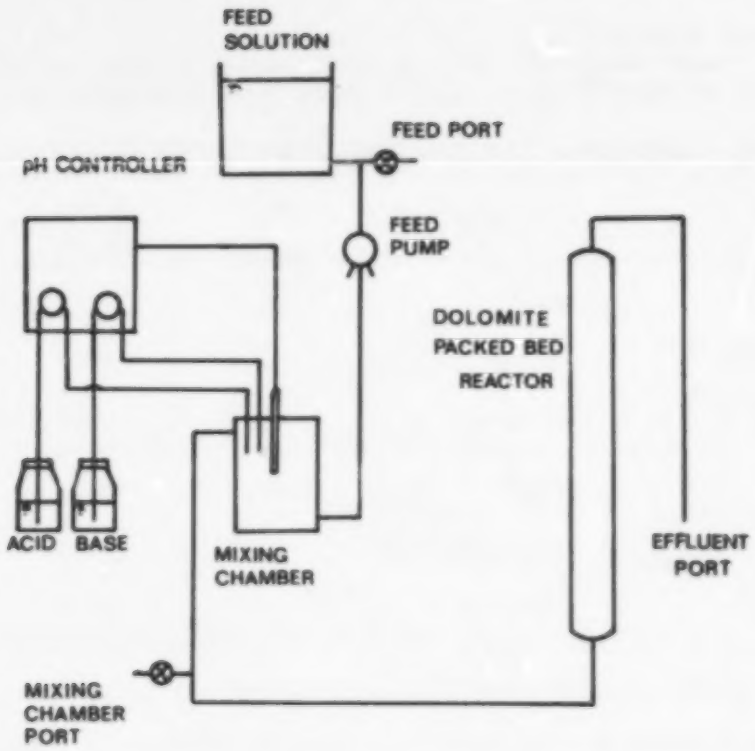


Figure 19. Schematic of continuous flow sulfide feed packed bed reactor system.

the packed bed reactors. The cultures adapted very readily to the sulfide electron donor. So readily in fact, that growth developed in the mixing chamber as well as in the dolomite reactor.

Steady-state data was collected for a period of twenty-six days. The reactor feed, mixing chamber and effluent were sampled daily and analyzed for nitrate, nitrite, pH, alkalinity, sulfate, and sulfide.

Experimental Results--

The steady-state data for the feed, mixing chamber and effluent solution of reactors in Experiment #1 of this study phase are summarized in Table 20.

TABLE 20. EXPERIMENT #1 - PHASE III STEADY-STATE EXPERIMENTAL RESULTS

	Sample point	NO_3^--N (mg/ℓ)	$S^=$-S (mg/ℓ)	NO_2^--N (mg/ℓ)
Expt. #1a (C_F = 3.1)	Feed	24.0	107.3	<0.05
	Mixing chamber	19.4	93.7	<0.05
	Effluent	1.1	20.4	<0.05
Expt. #1b (C_F = 0.96)	Feed	21.2	29.1	<0.05
	Mixing Chamber	13.8	4.34	8.2
	Effluent	<0.5	15.6	<0.05

This data is the mean of seven days of steady-state operation.

In Experiment #1b the feed solution was switched to a feed ratio of 0.96 after an extended period of operation under nitrate limiting growth conditions ($C_F \cong 3.1$). In the mixing chamber there was a significant accumulation of nitrite which is indicative of electron donor limiting growth conditions. In the effluent, however, nitrate and nitrite removals were essentially complete.

Experiment #2 - Elemental Sulfur Packed Bed Reactors

Experimental Plan and Techniques--

In Experiment #2 elemental sulfur was used as both the packing media and the electron donor in three continuous flow packed bed reactors. Each of these reactors contained different size sulfur particles as shown in Table 21. The reactors were operated at a series of hydraulic retention times ranging from 2.8 to 18.1 hours. A schematic of the units is shown in Figure 20. A synthetic waste containing 50 mg/ NO_3^--N (see Table 19) was passed in an upflow mode through these columns.

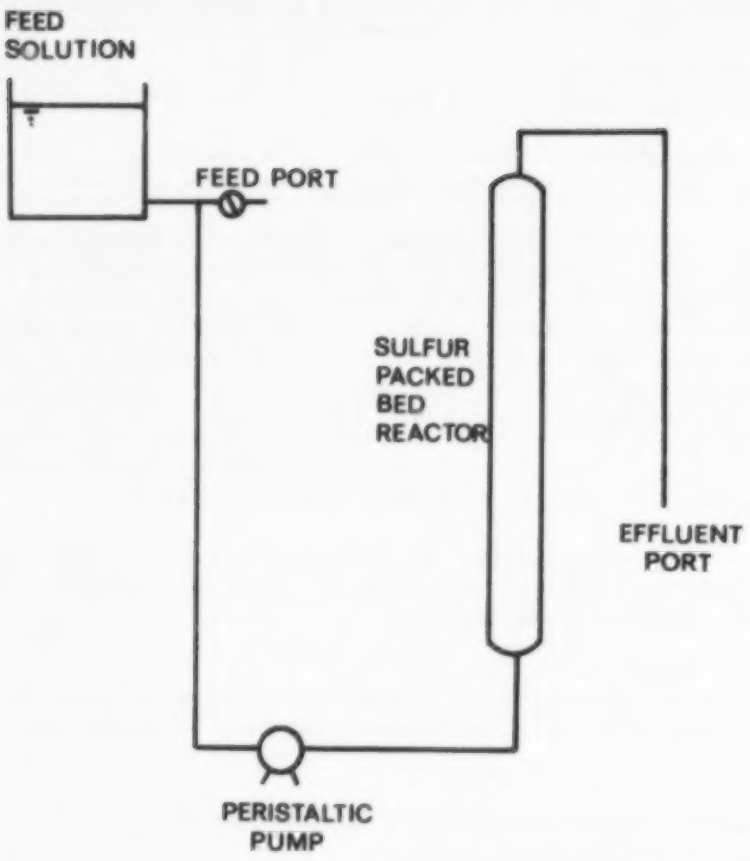


Figure 20. Schematic of continuous flow sulfur packed bed reactor system.

TABLE 21. PHASE III EXPERIMENT #2 REACTOR CHARACTERISTICS

Sulfur particle size range (mm)	13 - 19	7 - 13	2 - 7
θ Range (hr)	8.1 - 18.1	7.1 - 15.2	2.8 - 11.3

Appreciable difficulty was experienced in developing cultures that would adhere to and metabolize the elemental sulfur particles. A solution to this problem was to introduce the culture to the sulfur column and add thiosulfate in the feed solution. With time the microorganism population developed to the point where attachment to the sulfur particle surface occurred. The readily available thiosulfate was, however, still being oxidized. When a visible culture had developed the thiosulfate was gradually eliminated from the feed solution in order to force the organisms to metabolize the elemental sulfur. Once the population became established no difficulties were encountered in metabolizing the elemental sulfur.

Each column was operated until steady-state conditions were reached. Column influent and effluent were sampled daily and analyzed for nitrate, nitrite, pH, alkalinity and sulfate.

Experimental Results--

The steady-state data for Experiment #2 of this phase is summarized in Figure 21. Each data point represents the mean of seven days steady-state data.

Experiment #3 - Sulfur-Dolomite Packed Bed Studies

Experimental Plan and Techniques--

In Experiment #3 of Phase III, two elemental sulfur columns similar to those used in Experiment #2 were employed. One of the reactors was supplemented with dolomitic limestone. This sulfur-dolomite reactor was operated at a mean hydraulic retention time of 20.2 hours, in an upflow mode as shown in Figure 20. The other column was packed with only elemental sulfur to be used as a control. The sulfur particle size range in this reactor was 7 to 13 mm. This reactor was operated at a mean hydraulic retention time of 15.8 hours. A description of the media used in these reactors is given in Table 22. The feed solution composition for Experiment #3 is listed in Table 19.

The reactors were started using the procedure described in Experiment #2 of this study Phase.

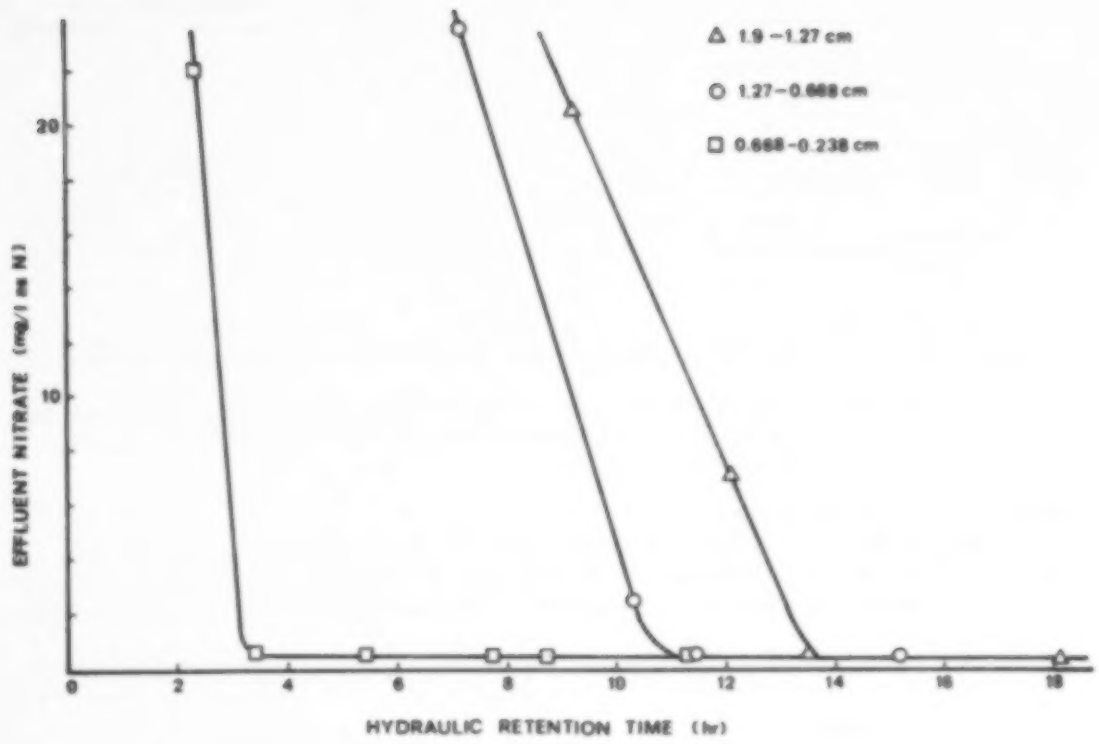


Figure 21. Effluent nitrate concentration as a function of hydraulic retention time for different particle size reactor media.

TABLE 22. PHASE III EXPERIMENT #3 REACTOR CHARACTERISTICS

	Sulfur media reactors	Sulfur-dolomite media reactors
Sulfur particle size range (mm)	7 - 13	2 - 7
Dolomite particle size range (mm)	-	2 - 13
$\frac{\text{Dolomite}}{\text{Sulfur}}$ mass ratio	0	0.357
Mean θ (hrs)	15.8	20.2

The columns were operated until steady-state conditions were reached. Column influent and effluent were sampled daily and analyzed for nitrate, nitrite, pH, alkalinity and sulfate.

Experimental Results--

The steady-state data for Experiment #3 of Phase III is given in Table 23 for the sulfur/dolomite system and in Table 24 for the sulfur system. Each data point represents the average of seven days steady-state operation.

TABLE 23. EXPERIMENT #3 - PHASE III RESULTS - SULFUR/DOLOMITE REACTOR

FEED			EFFLUENT			
Alk (mg/ℓ as $CaCO_3$)	pH	NO_3^--N (mg/ℓ)	Alk (mg/ℓ as $CaCO_3$)	pH	NO_3^--N (mg/ℓ)	NO_2^--N (mg/ℓ)
309	8.1	50.5	267	7.2	<0.5	<0.05
220	7.5	49.8	219	7.1	<0.5	<0.05
184	7.6	49.3	204	7.1	<0.5	<0.05
97	7.3	48.5	162	7.3	<0.5	<0.05
37	7.0	47.0	128	7.0	<0.5	<0.05

TABLE 24. EXPERIMENT #3 - PHASE III RESULTS - SULFUR REACTOR

FEED			EFFLUENT			
Alk (mg/ℓ as $CaCO_3$)	pH	NO_3^--N (mg/ℓ)	Alk (mg/ℓ as $CaCO_3$)	pH	NO_3^--N (mg/ℓ)	NO_2^--N (mg/ℓ)
309	8.1	50.5	244	6.8	<0.5	<0.05
257	7.6	47.7	131	6.5	28.9	13.7
220	7.5	49.8	67	6.2	11.0	9.0

Experiment #4 Packed Bed Studies with Domestic Wastewater Effluent

Experimental Plan and Techniques--

In the final experiment, Experiment #4, of Phase III, effluent from a secondary domestic wastewater treatment plant was used as feed to two packed bed reactors. The study employed two columns from Experiment #3. One of the columns contained only sulfur (particle size range 7 to 13 mm). The second column was packed with sulfur and dolomite (particle size range 7 to 13 mm).

The feed to these columns was gradually changed from the synthetic feed of Experiment #3 to secondary effluent. The secondary effluent had an average COD of 68 mg/ℓ, an alkalinity of 188 mg/ℓ as $CaCO_3$, a pH of 7.2 and a nitrate-nitrogen of 2.5 mg/ℓ. Since the effluent was not nitrified, supplemental nitrate was added to bring the total NO_3^--N concentration to 25 to 30 mg/ℓ.

The sulfur/dolomite column was operated in an upflow continuous flow mode with a hydraulic retention time of 27.8 hours. The sulfur column was operated in a similar manner with a hydraulic retention time of 21.1 hours.

The columns were operated for 26 days. Column influent and effluent were sampled daily and analyzed for alkalinity, pH, nitrate, nitrite, sulfate and COD.

Experimental Results--

The steady-state data for Experiment #4 of Phase III is given in Tables 25 and 26. Each data point is the mean of 14 days of steady-state operation.

TABLE 25. EXPERIMENT #4 - PHASE III
SULFUR/DOLOMITE PACKED BED REACTOR PERFORMANCE WITH
SECONDARY EFFLUENT FEED (θ = 27.8 HRS)

Parameter	Influent (mg/ℓ)	Effluent (mg/ℓ)
Alkalinity (as $CaCO_3$)	205	201
NO_2^--N	not detect.	0.015
NO_3^--N	27	0.1
$SO_4^=$-S	55	183
COD	68	32

TABLE 26. EXPERIMENT #4 - PHASE III
SULFUR PACKED BED REACTOR PERFORMANCE WITH
SECONDARY EFFLUENT FEED (θ = 21.1 HRS)

Parameter	Influent (mg/ℓ)	Effluent (mg/ℓ)
Alkalinity (as $CaCO_3$)	269	261
NO_2^--N	not detect.	<.05
NO_3^--N	27	0.1
$SO_4^=$-S	55	183
COD	68	40

Discussion of Phase III Experimental Results

Packed Bed Performance with Sulfide Electron Source--

The results of Experiment #1a of Phase III show that complete denitrification can be accomplished using sulfide as an electron donor, in dolomite packed beds, under nitrate limiting growth conditions (C_F = 3.1). The data shown in Table 20 shows, however, that even under electron donor limiting conditions, nitrate removal can be complete. This table shows that there was a significant accumulation of nitrite in the mixing chamber which is indicative of electron donor limiting growth conditions. In the effluent, however, nitrate and nitrite removals were complete. The ability of the reactor to maintain complete nitrate removal under apparently sulfide limiting growth conditions was probably due to utilization of elemental sulfur that accumulated within the packed bed under the previous nitrate limiting growth conditions. This elemental sulfur was probably the result of a partial oxidation of the feed sulfide in Experiment #1a. Such an observation indicates that this system would have stability under fluctuating feed ratio (C_F) conditions. However, if the system was operated for prolonged periods of time under electron donor limiting growth conditions the sulfur that accumulated within the reactor would diminish and nitrate removals would deteriorate.

Elemental Sulfur Packed Bed Performance--

The data for Experiment #2 of this Phase (Figure 21) shows that complete denitrification was obtained with sulfur as the electron donor provided that a minimum hydraulic retention time was provided. This minimum hydraulic retention time appears to be a function of the sulfur particle size in the reactor.

An example of the data obtained for one of the Experiment #2 reactors is shown in Figure 22. This figure is a plot of effluent quality vs hydraulic retention time. At long hydraulic retention times nitrate removal is essentially complete, sulfate concentration in the effluent is high, and alkalinity concentration is low relative to the feed concentration. This characterizes a system that is functioning properly. As the hydraulic retention time is decreased, a point will be approached at which the system is stressed. When a system reaches a minimum hydraulic retention time there will be an increase in effluent nitrate and nitrite, a decrease in effluent sulfate and an increase in effluent alkalinity as shown in Figure 22.

It is evident from Figure 21 that each reactor with a different sulfur particle size has a different minimum hydraulic retention time. The minimum hydraulic retention time required for complete nitrate removal decreases with decreasing sulfur particle size.

If the sulfur particles in the reactor are assumed to be spherical, with an average diameter calculated from the sieve size analysis, it is possible to estimate the sulfur surface area in the reactor. The estimated sulfur surface area may then be plotted against the minimum hydraulic retention time for each reactor, as shown in Figure 23. There appears to be strong correlation between reactor sulfur surface area and the minimum hydraulic retention time required for complete nitrate removal. This suggests that in the

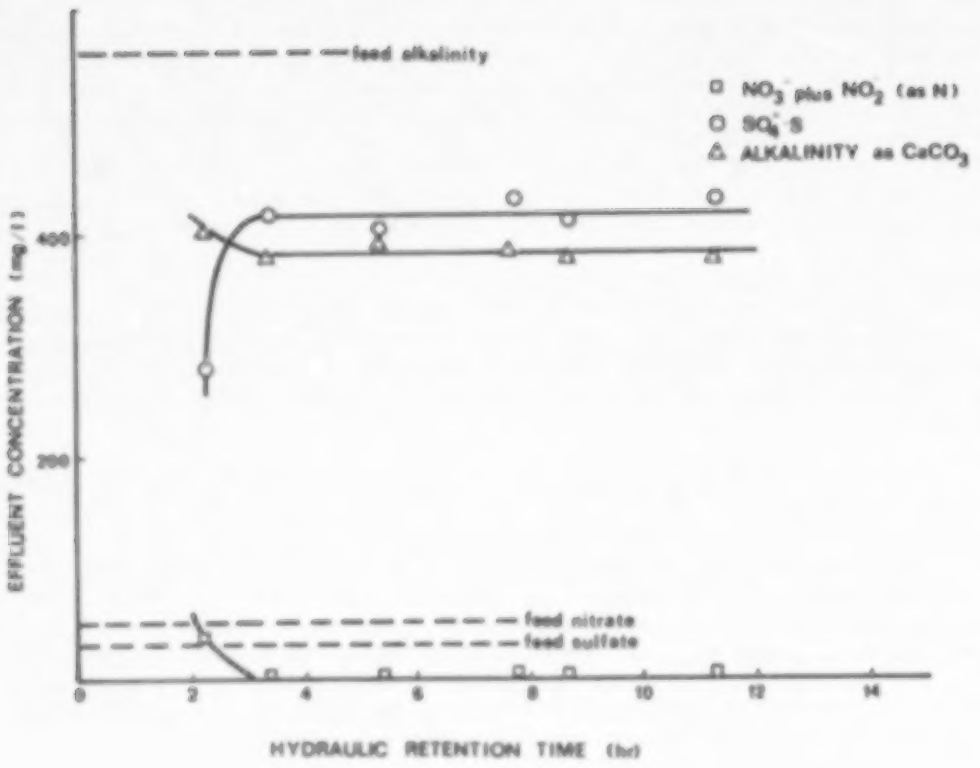


Figure 22. Effluent quality as a function of hydraulic retention time.

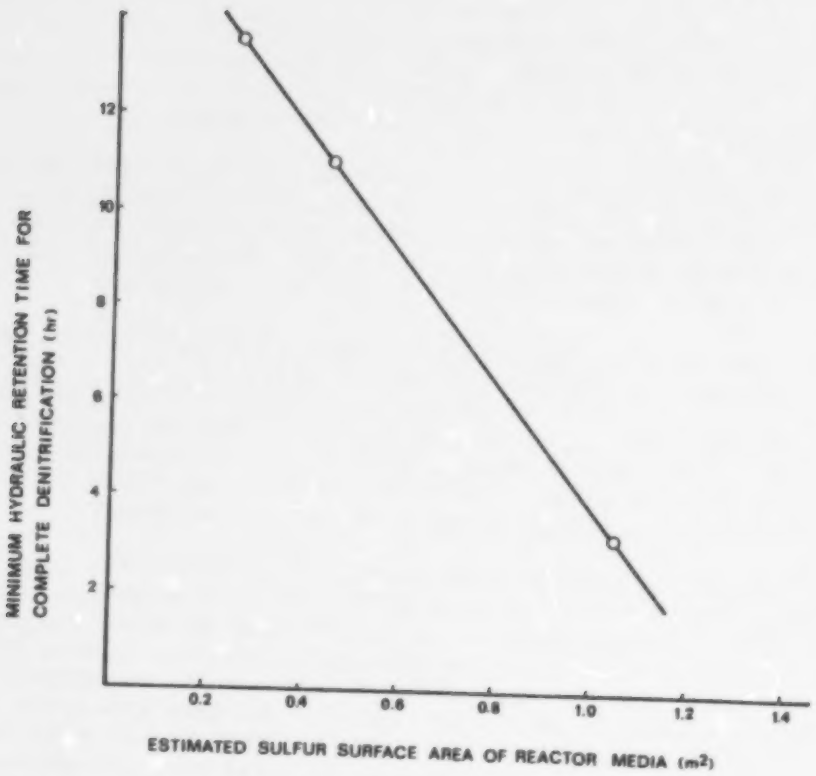


Figure 23. Minimum hydraulic retention time for complete denitrification as a function of estimated sulfur surface area.

design of a sulfur media packed bed reactor, sulfur surface area is a major consideration.

Alkalinity Supplementation with Dolomite--

The intent of Experiment #3 of Phase III was to examine the extent to which dolomitic limestone could supply alkalinity to the denitrifying cultures. Inorganic carbon, in the form of biocarbonate and carbonate usually buffer these systems against the metabolic addition of hydrogen ion. Inorganic carbon is also used by the denitrifiers as a cellular carbon source. From Equations 20 and 21 the amount of inorganic carbon required for denitrification biomass synthesis is computed to be 1.07 mg C per mg of NO_3^--N reduced. The amount of alkalinity consumed is computed to be 4.38 mg of alkalinity as $CaCO_3$ per mg NO_3^--N reduced. From these theoretical calculations it appears that the system buffer capacity will normally be exceeded long before inorganic carbon growth limiting conditions will develop.

To verify the predicted alkalinity consumption rates, and determine how much alkalinity could be supplemented by dolomite, both reactors, in Experiment #2 were initially supplied with substantial alkalinity (in the form of sodium bicarbonate) in amounts well above that theoretically required. The feed alkalinity was then gradually reduced in both systems.

In the system with no dolomite supplement the pH was reduced to 6.5 and 6.2 with average feed alkalinities of 257 mg/ℓ as $CaCO_3$ and 210 mg/ℓ as $CaCO_3$, respectively. In both cases denitrification efficiency was greatly reduced and nitrite accumulation became apparent. Some acclimation of the microorganisms to these low pH values, however, was noted as the systems were operated beyond the test period. Baalsrud and Baalsrud (58) suggest that the lower pH limit for Thiobacillus denitrificans is approximately 6.2.

In the packed bed reactor, with dolomite supplement, the feed alkalinity was lowered from 309 to 37 mg/ℓ as $CaCO_3$ with no significant pH depression or denitrification hinderance. It appears that dolomite has the ability to supply significant alkalinity when the feed alkalinity is very low. The amount of alkalinity that is supplied by the dolomite in an actively denitrifying reactor is a function of the feed nitrate and feed alkalinity concentrations. From the experimental data it appears that the dolomite supplies enough alkalinity to compensate for biological alkalinity comsumption plus an additional amount which is controlled by solubility phenomena. Apparent alkalinity consumption is determined by the difference between feed and effluent alkalinity concentration. When the feed alkalinity becomes low enough the packed bed reactor with dolomite experienced negative apparent alkalinity consumption (alkalinity production). This is demonstrated in Figure 24. An explanation of this phenomena is that in high alkalinity feed solutions the concentration gradient between the dolomite surface and bulk solution is low, hence dissolution of limestone is not significant. However, when the concentration gradient is high, as in the case of low alkalinity waters, dissolution of limestone is enhanced.

It is not possible to verify the predicted alkalinity consumption in

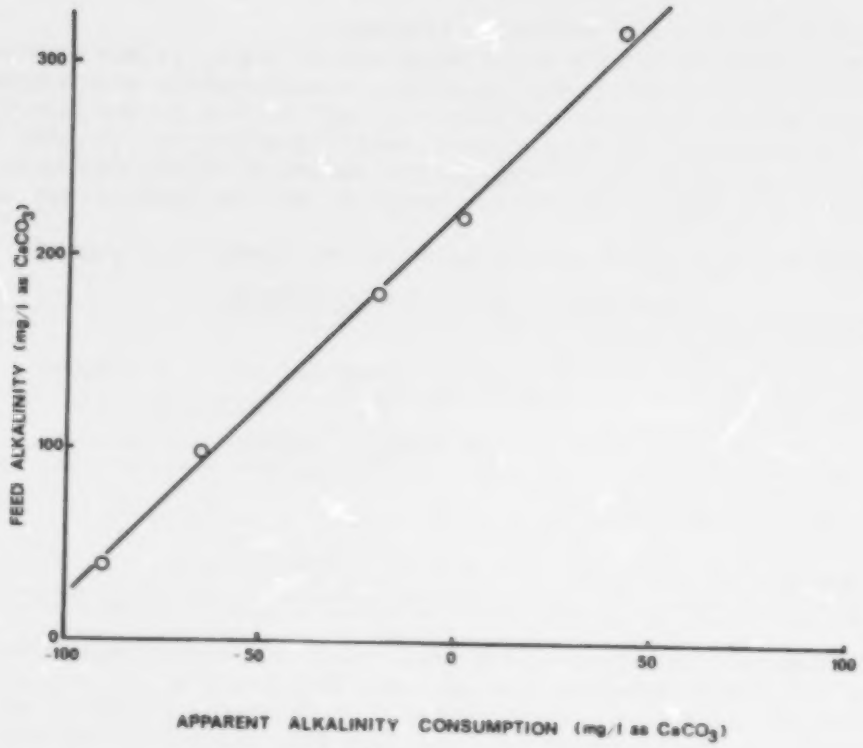


Figure 24. Feed alkalinity versus apparent alkalinity consumption for sulfur packed bed reactors supplemented with dolomite.

the dolomite supplemented systems. However, in the sulfur column reactors in both Experiment #2 and #3 the amount of alkalinity consumed (as $CaCO_3$) per amount of NO_3^--N reduced averaged 3.7. This compares well with the theoretical value of 4.4 when the low accuracy and precision of the alkalinity analysis is considered.

Although the use of dolomite seems to be a cheap and effective method to compensate for alkalinity consumption in the autotrophic denitrification process, it has the disadvantage of increasing the hardness of effluents. In most cases, the incremental hardness is not a significant consideration.

Denitrification of Domestic Secondary Effluent--

In Experiment #3 of this third experimental phase, it was apparent that heterotrophic denitrification was proceeding simultaneously with autotrophic denitrification when secondary effluent was used as feed to the packed bed reactors. The presence of heterotrophic denitrification is indicated by the relatively low amount of $SO_4^=$-S produced per amount of NO_3^--N reduced as shown in Table 27. This table compares the amount of sulfate produced per amount

TABLE 27. A COMPARISON OF MEASURED AND THEORETICAL SULFATE PRODUCTION TO NITRATE REDUCTION RATIO

	mg $SO_4^=$-S produced per mg NO_3^--N reduced	
	Theoretical	Measured, secondary effluent
Sulfur/dolomite	7.6	4.7
Sulfur	7.6	4.7

of nitrate reduced for a theoretical prediction (no organic matter in feed), and for the secondary effluent feed system. The amount of $SO_4^=$-S produced per amount of NO_3^--N reduced can be calculated from Equations 20 and 21 to be 7.6. The results of Experiment # 4 show that this parameter averages to be 4.7 mg $SO_4^=$ -S per mg NO_3^--N reduced. It appears, therefore, that organic matter was being utilized as an electron donor, as well as sulfur.

No problems were encountered with biological production of sulfide when secondary effluent was used as the feed to the packed bed systems. No suspended solids clogging was experienced during any of the experiments.

SECTION 8

ENGINEERING SIGNIFICANCE

Autotrophic denitrification using sulfur compounds provides an alternative method for biological denitrification. Based on results of these bench-top studies, the process appears to offer advantages of decreased cost of electron donor while being independent of rising petrochemical prices. However, autotrophic denitrification is not without disadvantages. It enriches the wastewater with sulfate and normally would require supplemental additions of alkalinity to offset microbial acid production.

EVALUATION OF SULFUR-SUBSTRATES

A wide variety of reduced sulfur compounds are potential substrates for autotrophic denitrification. Evaluation of the relative merits of these compounds involves estimation of cost and consideration of ease of storage and handling. The relative cost of various sulfur substrates depends on their initial cost of purchase and the stoichiometric amount of the compound required for nitrate removal. The exact amount required depends on unknown reaction stoichiometry, but since nitrate removal is accomplished by a microbially mediated oxidation-reduction reaction, a reasonable comparison of the sulfur compounds can be made on an electron equivalent basis. Table 28 shows a comparison of the cost of various sulfur substrates and methanol expressed on an electron equivalent basis. An electron equivalent is that quantity of a compound which donates or accepts one mole of electrons in the oxidation-reduction reaction in question. For example, consider the half-reaction involved in the oxidation of elemental sulfur by nitrate.

$$\text{Oxid.} : 0.167\ S + 0.667\ H_2O \rightarrow 0.167\ SO_4^{=} + 1.33\ H^+ + e^-$$

$$\text{Red.} : 0.2\ NO_3^- + 1.2\ H^+ + e^- \rightarrow 0.10\ N_2 + 0.6\ H_2O$$

$$\text{Overall:}\ 0.167\ S + 0.2\ NO_3^- + 0.067\ H_2O \rightarrow 0.10\ N_2 + 0.167\ SO_4^{=} + 0.13\ H^+$$

Since these reactions are written on the basis of one electron transfers, it is simple to calculate the electron equivalents of sulfur and nitrate. One electron equivalent of nitrate will be 0.2 moles of nitrate, or 0.28 grams of nitrate-nitrogen. One electron equivalent of sulfur will be 0.167 moles of sulfur, or 5.33 grams of sulfur. Therefore, the electron content of elemental sulfur will be 1 electron equivalent/5.33 grams = 0.188 electron equivalents/gram.

TABLE 28. COMPARISON OF COSTS OF SULFUR SUBSTRATES AND METHANOL**

Compound	Unit cost* ($/100 lbs)	Electron content (electron-equivalents/gram)	Cost/electron equivalent (¢/electron equivalent)
Anhydrous sodium sulfite	11	1.58×10^{-2}	1.53
Sodium thiosulfate pentahydrate	8.30	3.23×10^{-2}	0.567
Sodium thiosulfate anhydrous	10.95	5.05×10^{-2}	0.477
Sodium sulfide flake	12.50	3.33×10^{-2}	0.826
Hydrogen sulfide	10	0.235	0.094
Sulfur crude flour	4.70	0.188	0.055
Methanol	7.59	0.188	0.089

* $(\$/10^2 \text{lbs}) \times (2.2 \times 10^{-2}) = (\$/\text{kg})$.

** Reference 59.

From Table 28, it is apparent that elemental sulfur is the least expensive sulfur source since it has the lowest initial cost and is relatively highly reduced. Hydrogen sulfide is more highly reduced but its higher initial cost makes it less attractive. Sulfite and thiosulfate are expensive due to high initial cost and low electron content. The high cost of these compounds, however, might be avoided if industrial wastes containing them are available.

Elemental sulfur has the additional advantage that it is easy to store and handle. It is non-toxic, water-insoluble and stable under normal conditions. A major disadvantage of sulfide is its toxicity. Storage and handling of sulfide would be more difficult than for sulfur since loss of sulfide through leaks in storage or effluent losses due to overdosing could cause a serious health or odor problem. Thiosulfate and sulfite should be relatively easy to store and handle but larger storage volumes would be required.

Elemental sulfur is produced primarily by Frasch process mining and recovery of sulfur compounds from natural gas, petroleum and coal (60,61). Sulfide can be produced by recovery from natural gas and petroleum (60), hydrogenation of elemental sulfur (61), and reduction of sulfate with coal (60,61). Sulfide can also be obtained as a constituent of various industrial wastes such as black liquor from Kraft paper mills (60). Methods for the commercial production of thiosulfate include reaction of elemental sulfur with sulfite and reaction of sulfide with sulfur dioxide (60). Thiosulfate can also be found in some industrial wastes such as those from petroleum refineries (62). Absorption of sulfur dioxide in alkaline solution is the primary method of sulfite production (60). Sulfur dioxide for this process can be obtained during burning elemental sulfur or roasting sulfidic ores (60,61). Wastewaters from sulfite-pulping operations or other bleaching processes also contain sulfite (60).

Another potential source of reduced sulfur compounds is recovery of sulfur from emissions of sulfur oxides at power plants burning coal. Two sulfur oxide removal processes, which have been certified as being ready for commercial use by 1978, produce a stream of concentrated sulfur dioxide (63). The sulfur dioxide in this stream could be reduced to elemental sulfur by reaction with coal (64). The potential magnitude of this source can be seen by comparing the estimated power plant emissions of sulfur dioxide in 1968 (12.2 million tons) to the total U.S. sulfur production for that year (10.4 million tons) (65,66).

TECHNICAL FEASILIBITY

The ultimate feasibility of any wastewater treatment process depends on its economic, social, and environmental costs relative to alternative processes. Accurate feasibility analyses, however, usually require pilot plant testing of the process. A less detailed analysis of the technical feasibility of a process can be based on the results of bench-top experiments. such as those conducted during this study. A technical feasibility analysis should determine whether a process can be expected to operate under reasonable technical constraints and whether pilot plant tests are warranted to

determine the feasibility of full scale operation.

Because heterotrophic denitrification using methanol is the most common denitrification process in present use (1978), it is reasonable to use it in a technical feasibility analysis as the standard of comparison for evaluating autotrophic denitrification using elemental sulfur. The elemental sulfur-slurry type bench-top studies on autotrophic denitrification reported here, were conducted using a hydraulic retention time of six hours, which is slightly higher than that used in heterotrophic processes (67). Heterotrophic processes, however, usually required and additional aeration basin to remove overdoses of organic carbon. Autotrophic processes would not require aeration since sulfur is a water-insoluble substrate and is recycled with biomass. Autotrophic processes can be operated at somewhat higher overflow rates than the value of 49 m^3/m^2-d (1200 gpd/ft^2) recommended for heterotrophic processes (68). Since the values of these parameters (θ, Q/A_c) are roughly comparable, autotrophic denitrification can be considered technically feasible with respect to both reactor and clarifier-thickener sizes. Pilot plant studies on autotrophic denitrification would be required to more fully evaluate equipment cost comparisons. Similarity in the requirements for capital expenditures indicates that the best criterion for comparison of the two processes is the operating cost of purchasing the electron donor.

COST OF ELECTRON DONOR

The cost of methanol required to treat a wastewater with 30 mg/ℓ NO_3^--N can be estimated as 1.2¢/m^3 ($0.046/1000 gal). This calculation is based on a bulk methanol price of $1.1¢/ℓ (69), and a methanol to nitrage-nitrogen feed ratio of 3.0 (67). Sulfur costs for treating the same wastewater by the autotrophic denitrification slurry process operating at S/X = 40 would be 1.7¢/m^3 ($0.066/1000 gal). This calculation is based on a feed ratio of 5.6 mg S/mg NO_3^--N, and a sulfur cost of $0.104/kg ($4.70/100 lb) (69).

The total sulfur requirement for a slurry system is equal to the amount of sulfur oxidized by the denitrification reaction plus the amount needed to replace that lost with solids wasting. Decreasing the amount lost with waste solids, increases the sulfur utilization efficiency. This is accomplished by operating at lower values of S/X but requires higher solids retention times because of slower growth rates. An upper limit on the efficiency of sulfur utilization is set by reaction stoichiometry (Equation 38) and is equivalent to a sulfur cost of 0.77¢/m^3 ($0.029/1000 gal.) for a wastewater with 30 mg/ℓ NO_3^--N. Operation at higher than normal values of θ_c may be possible since there appear to be no thickening limitations and transfer of electron acceptor is not such a problem as in aerobic systems. However, extrapolation of the kinetic and solids separation data obtained in this study to lower values of S/X should be done with caution.

Maximum sulfur utilization efficiency can be approached using a packed bed configuration for autotrophic denitrification. This configuration does not require wasting of sulfur as in slurry systems. However, the larger sulfur particles used would require larger reactor volumes. In this study, hydraulic retention times in the packed bed reactors were 3 to 4 times as

long as those used in the slurry reactors. Hydraulic retention time is not a primary design parameter for slurry systems, but the values used in this study are probably representative of those that would be adopted for full scale operation.

Dependence of process economics on the cost of sulfur indicates the usefulness of a review of recent sulfur market trends. Within the last two years, the cost of sulfur has ranged from a high of $0.12/kg ($5.60/100 lb) to a low of $0.055/kg ($2.50/100 lb) (70). The cost advantage of a heterotrophic slurry system using methanol would be reversed if the lower price had remained stable at 0.42 vs 1.22¢/m^3, for sulfur and methanol, respectively. The comparison is more strongly in favor of autotrophic denitrification if the minimum sulfur requirement of 2.52 mg S/mg NO_3^--N is used; 0.42 vs 1.22¢/m^3 ($0.016 vs $0.046/1000 gal), sulfur vs methanol, respectively. Economic feasibility of autotrophic denitrification appears to be closely linked to the behavior of the sulfur and methanol markets. It is possible that the future cost of sulfur will decrease if sulfur recovery processes for stack gases become widespread.

COST OF SUPPLEMENTAL ALKALINITY

Approximately 0.092 milliequivalents of alkalinity are destroyed per milligram nitrate-nitrogen removed by autotrophic denitrification (Table 15). A wastewater containing 30 mg/ℓ NO_3^--N would require replenishment of 2.75 meq/ℓ alkalinity costing 0.32¢/m^3 ($0.014/1000 gal) at a lime cost of $0.0358/kg ($32.50/ton) (69). Lime addition should be made prior to the nitrification step in order to aid nitrification by raising the pH, and to convert the added alkalinity to carbonate alkalinity. If this were done, addition of 2.75 meq/ℓ alkalinity would be equivalent to 33 mg/ℓ inorganic carbon. This will easily satisfy the carbon requirement of the autotrophic organisms of 10.3 mg/ℓ C. This value was calculated using the balanced stoichiometric equation (Equation 38) and assuming that 30 mg/ℓ NO_3^--N would be completely denitrified.

In the packed bed reactor configuration dolomite provides a cheaper alkalinity supplement.

SLUDGE DISPOSAL

No experimental data on the behavior of a sulfur-biomass slurry in waste solids treatment processes are available. Probable behavior can be deduced by estimating the effect of the high sulfur content of the slurry. The sulfur particles should give the slurry a stronger structure, aiding dewatering by vacuum filtration. Anaerobic digestion could be used to decrease the organic fraction of the solids. Incineration of the sludge would not be feasible because of the production of large amounts of sulfur dioxide. Aerobic digestion would require large amounts of alkalinity addition and would produce an effluent high in sulfates and dissolved solids. Ultimate landfill of the residue should be practiced with caution because of the large potential for production of acid in the sulfur-biomass sludge.

In the packed bed systems sulfur does not leave the columns other than in the $SO_4^=$ form. Hence, sludge disposal is not a problem.

ENVIRONMENTAL IMPACT

Sulfur oxidation in the autotrophic denitrification process will enrich the wastewater in sulfate. Increased sulfate levels might decrease water quality directly or through the potential for sulfide production. Reaction stoichiometry predicts an increase of 75 mg/ℓ $SO_4^=$-S in a wastewater containing 30 mg/ℓ NO_3-N. An average domestic wastewater contains about 25 mg/ℓ $SO_4^=$-S, (38,71) so most of these wastewaters would not meet the old drinking water standard of 83 mg/ℓ $SO_4^=$-S (72) after denitrification with elemental sulfur. The old standard does not appear to be based on taste or on any other physiological basis, except for a laxative effect on new users (73). Present U.S. drinking water standards do not contain a sulfate regulation (74). Therefore, a sulfate concentration of about 100 mg/ℓ $SO_4^=$-S should not cause serious deterioration of drinking water quality, especially when dilution, effects are considered.

Certain microorganisms can reduce sulfate to sulfide in natural water systems if sufficient organic carbon is available under anaerobic conditions. Production of sulfides cause an odor problem and precipitation of heavy metals. Insolubilization of some highly toxic metals such as mercury would be beneficial. Formation of metallic sulfides could theoretically cause release of phosphorus previously immobilized by the metal. Experimentation, however, has not shown this to occur (75).

Nitrogen removal is especially important in coastal and estuarine waters where the effects of sulfate enrichment would be negligible due to interactions with seawater containing high concentrations of sulfate (900 mg/ℓ $SO_4^=$-S) (76). Although sulfate enrichment does not improve water quality, there does not seem to be sufficient evidence of harmful effects to generally restrict the application of autotrophic denitrification processes.

SUMMARY

In summary, autotrophic denitrification compares favorably with presently employed heterotrophic denitrification processes. At this time (1978), elemental sulfur appears to be a lower cost substrate than methanol. The cost of supplemental alkalinity is not excessive. Another advantage of autotrophic denitrification is that sulfur addition does not require precise control, since overdoses will not appear in the effluent. Development of new sludge handling techniques does not appear to be necessary for elemental sulfur-biomass slurries. Further, the problem of sulfate enrichment does not appear to be serious enough to restrict application of autotrophic denitrification. Therefore, autotrophic denitrification using elemental sulfur appears to be a technically feasible wastewater treatment process and should be evaluated in pilot plant or full scale studies.

REFERENCES

1. Baalsrud, K., K. S. Baalsrud, "Studies on Thiobacillus Denitrificans", Arch. Microbiol., 20:34-62, 1954.

2. Buchanan, R. E., N. E. Gibbons, eds., Bergey's Manual of Determinative Bacteriology, 8th Ed., Williams and Wilkins, Co., Baltimore, 1974.

3. Verhoeven, W., "Studies on True Dissimilatory Nitrate Reduction: V Nitric Oxide Production and Consumption by Micro-organisms", Antonie Van Leeuwenhoek, 22:383-406, 1956.

4. T'yul'panova-Mosevich, M. V., "Denitrifikatsiya na Neorganicheskoi Srede (Denitrification in Inorganic Media)", Arkhiv Biologicheskikle Nauk, 30(2):---, 1930, reported in Sokalova, G. A., G. I. Karavaiko, Physiology and Geochemical Activity of Thiobacilli, Israel Program of Scientific Translation, Jerusalem, 1968.

5. Kramarenko, L. E., I. I. Prisronova, "Denitrifying Sulfur Oxidizing Bacteria in Sulfide Veins and Methods of Demonstrating Them in Prospecting", Proc. All-Union Geol. Res. Inst. USEGEI, 61:209-230, 1961, reported in Buchanan, R. E., N. E. Gibbons, eds., Bergey's Manual of Determinative Bacteriology, 8th Ed., Williams and Wilkins Co., Baltimore, 1974.

6. Pan, P. C., "Growth of Three Obligate Autotrophs, Thiobacillus Thioparus, Thiobacillus Neopolitanus, Thiobacillus Denitrificans, on Glucose", Bacteriol. Proc., 70:125, 1970.

7. Borichewski, R. M., "Keto Acids as Growth Limiting Factors in Autotrophic Growth of Thiobacillus Thiooxidans", J. Bacteriol., 93(2): 597-599, 1967.

8. Karavaiko, G. I., S. A. Moshniakova, "Oxidation of Sulphide Minerals by Thiobacillus Thiooxidans", Microbiology, 43(1):156-158, 1974.

9. Taylor, B. F., D. S. Hoare, S. L. Hoare, "Thiobacillus Denitrificans as an Obligate Chemolithotroph: Isolation and Growth Studies", Arch. Microbiol., 78(3):193-204, 1971.

10. Suzucki, I., "Mechanism of Inorganic Oxidation and Energy Coupling", Annu. Rev. Microbiol., 28:85-119, 1974.

11. Peck, H. D., "Sulfur Requirements and Metabolism of Microorganisms", in Muth, O. H., ed., Symposium: Sulfur in Nutrition, AVI Publ. Co., Westport, Conn., 1970.

12. Trudinger, P. A., "The Metabolism of Inorganic Sulphur Compounds by Thiobacilli", Rev. Pure Appl. Chem., 17:1-24, 1967.

13. Bowen, T. J., F. C. Happold, B. F. Taylor, "Studies on Adenosine-5' Phosphosulphate Reductase from Thiobacillus Denitrificans", Biochim. Biophys. Acta, 118:566-576, 1966.

14. Bowen, T. J., P. J. Butler, F. C. Happold, "Some Properties of the Rhodanase System of Thiobacillus Denitrificans", Biochem. J., 97:651-657, 1965.

15. Suzucki, I., "Oxidation of Elemental Sulfur by an Enzyme System of Thiobacillus Thiooxidans", Biochim. Biophys. Acta, 104(2):359-371, 1965.

16. Baldensperger, J.., L. J. Guarraia, W. J. Humphreys, "Scanning Electron Microscopy of Thiobacilli Grown on Colloidal Sulfur", Arch. Microbiol., 99(4):323-329, 1974.

17. Ishaque, M., M. I. H. Aleem, "Intermediates of Denitrification in Thiobacillus Denitrificans", Bacteriol. Proc., 72:175, 1972.

18. Adams, C. A., G. M. Warnes, D. J. D. Nicholas, "A Sulphite-Dependent Nitrate Reductase from Thiobacillus Denitrificans", Biochim. Biophys. Acta, 235:398-406, 197.

19. Aminuddin, M., D. J. D. Nicholas, "Sulphide Oxidation Linked to the Reduction of Nitrate and Nitrite in Thiobacillus Denitrificans", Biochim. Biophys. Acta, 325(1):81-93, 1973.

20. Aminuddin, M., D. J. D. Nicholas, "An AMP Independent Sulphite Oxidase from Thiobacillus Denitrificans: Purification and Properties", J. Gen. Microbiol., 82:103-113, 1974.

21. Aminuddin, M., D. J. D. Nicholas, "Electron Transport during Sulphide and Sulphite Oxidation in Thiobacillus Denitrificans", J. Gen. Microbiol., 82:115-123, 1974.

22. Peeters, T., M. I. H. Aleem, "Oxidation of Sulfur Compounds and Electron Transport in Thiobacillus Denitrificans", Arch. Microbiol., 71(4):319-330, 1970.

23. Peeters, T., M. Ishaque, M. I. H. Aleem, "Tetrathionate Oxidation in Thiobacillus Denitrificans", Bacteriol. Proc., 73:194, 1973.

24. Aubert, J. P., G. Milhaud, J. Millet, "L'Assimilation de L'Andydride Carbonique par les Bacteries Chimautotrophies", Ann. Inst. Pasteur, Paris, 92:515-524, 1957.

25. Peeters, T., M. S. Liu, M. I. H. Aleem, "The Tricarboxylic Acid Cycle in Thiobacillus Denitrificans and Thiobacillus A2", J. Gen. Microbiol.,

65(1): 29-35, 1970.

26. Kelly, D. P. "Autotrophy: Concepts of Lithotropic Bacteria and Their Organic Metabolism", Bacteriol. Rev., 25:177-270, 1971.

27. Gram, A. L., "Feasibility of Bacterial Reduction of Nitrates in Sulfur Columns", Final Report prepared for Federal Water Pollution Control Administration, Dept. of the Interior, Washington, D. C., Contract No. 14-12-125 (1968).

28. Smith, J. M., Chemical Engineering Kinetics, McGraw-Hill, New York, 1970.

29. Levenspiel, O., Chemical Reaction Engineering, 2nd Ed., Wiley, New York 1972.

30. Dick, R. I., "Evaluation of Activated Sludge Thickening Theories", J. Sanit. Eng. Div. Am. Soc. Civ. Eng., 93(SA4):9-29, 1967.

31. Vesilind, P. A., "Design of Prototype Thickeners from Batch Settling Tests", Water and Sewage Works, 115(7):302-307, 1968.

32. Clark, J. W., W. Viessman, M. J. Hammer, Water Supply and Pollution Control, 2nd Ed., International Textbook Co., Scranton, Pa. 1971.

33. McCarty, P. L., "Stoichiometry of Biological Reactions", in Proceeding of the International Conference Toward a Unified Concept of Biological Waste Treatment, Atlanta, Ga., 1972.

34. Lawrence, A. W., P. L. McCarty, "A Unified Basis for Biological Treatment Design and Operation", J. Sanit. Eng. Div. Am. Soc. Civ. Eng., 96 (SA3):757-778, 1970.

35. Heukelekian, H., H. E. Orford, R. Manganelli, "Factors Affecting the Quantity of Sludge Production in the Activated Sludge Process", Sew. and Ind. Wastes, 23(8):945-958, 1951.

36. Weston, R. F., W. W. Eckenfelder, "Applications of Biological Treatment to Industrial Wastes: I Kinetics and Equilibria of Oxidative Treatment", Sew. and Ind. Wastes, 27(7):802-820, 1955.

37. van Uden, N., "Kinetics of Nutrient Limited Growth", Annu. Rev. Microbiol., 23:473-486, 1969.

38. Metcalf & Eddy, Inc., Wastewater Engineering, McGraw-Hill, New York 1972.

39. Sherrard, J. H., E. D. Schroeder, "Cell Yield and Growth Rate in Activated Sludge", J. Water Pollut. Control Fed., 45(9):1889-1897, 1973.

40. Sherrard, J. H., L. D. Benefield, "Elemental Distribution Diagrams for Biological Wastewater Treatment", J. Water Pollut. Control Fed., 48(3):562-569, 1976.

41. McCarty, P. L., "Energetics and Bacterial Growth", in Organic Compounds in Aquatic Environments, S. D. Faust, J. V. Hunter, eds., Marcel Decker, Inc., New York, 1971.

42. U. S. Environmental Protection Agency, Methods Development and Quality Assurance Research Laboratory, NERC, Cincinnati, Methods for Chemical Analysis of Water and Wastes, 1974.

43. Standard Methods for the Examination of Water and Wastewater, 13th Ed., American Public Health Association, Washington, D. C., 1971.

44. Nelson, P. O., Adenosine Triphosphate as a Measure of Activated Sludge Viability, M. S. Thesis, Dept. of Civil and Environmental Engineering, Cornell University, May 1973.

45. Stumm, W., J. J. Morgan, Aquatic Chemistry, Wiley-Interscience, New York, 1970.

46. Stensel, H. D., R. C. Loehr, A. W. Lawrence, "Biological Kinetics of Suspended-Growth Denitrification", J. Water Pollut. Control Fed., 45 (2):249-261, 1973.

47. Porges, N., L. Jasewicz, S. R. Hoover, "Principles of Biological Oxidation", in Biological Treatment of Sewage and Industrial Wastes, Vol. I: Aerobic Oxidation, J. McCabe, W. W. Eckenfelder, Eds., Reinhold Pub. Co., New York, 1956.

48. Johnson, W. K., G. J. Schroepfer, "Nitrogen Removal by Nitrification and Denitrification", J. Water Pollut. Control Fed., 36(8):1015-1036, 1964.

49. Seidel, D. F., R. W. Crites, "Evaluation of Anaerobic Denitrification Processes", J. Sanit. Eng. Div. Am. Soc. Civ. Eng., 92(SA2):267-277, 1970.

50. Mechalas, B. J., P. M. Allen, W. W. Matyskiela, A Study of Nitrification and Denitrification, Federal Water Quality Administration, Dept. of the Interior, Washington, D. C., 1970.

51. Sikora, L. J., D. R. Keeney, "Evaluation of a Sulfur-Thiobacillus Denitrificans Nitrate Removal System", in press, J. Environ. Qual.

52. Requa, D. A., E. D. Schroeder, "Kinetics of Packed-Bed Denitrification", J. Water Pollut. Control Fed., 45(8):1696-1707, 1973.

53. Moore, S. F., E. D. Schroeder, "The Effect of Nitrate Feed Rate on Denitrification", Water Res., 5:445-452, 1970.

54. Engberg, D. J., E. D. Schroeder, "Kinetics and Stoichiometry of Bacterial Denitrification as a Function of Cell Residence Time", Water Res., 9(12):1051-1054, 1975.

55. Starkey, R. L., "Concerning the Physiology of Thiobacillus Thiooxidans, an Autotrophic Bacterium Oxidizing Sulfur Under Acid Conditions", J. Bacteriol., 10:135-195, 1925.

56. Dick, R. I., K. W. Young, "Analysis of Thickening Performance of Final Settling Tanks", Proc. Ind. Waste Conf., 27:33-54, 1972.

57. Batchelor, B., Autotrophic Denitrification Using Sulfur Electron Donors, Ph.D. Thesis, Dept. of Civil and Environmental Engineering, Cornell University, September, 1976.

58. Baalsrud, K., K. S. Baalsrud, "Studies on Thiobacillus Denitrificans" Arch. Microbiol., 20:34-62, 1954.

59. Chemical Marketing Reporter, September 29, 1975, reported in Driscoll, C. T., Use of Thiosulfate and Sulfide as Electron Donors in Autotrophic Denitrification, M. S. Thesis, Dept. of Civil and Environmental Engineering, Cornell University, September 1976.

60. Shreve, R. N., The Chemical Process Industries, McGraw-Hill, New York, 1956.

61. Sittig, M., Inorganic Chemical and Metallurgical Process Encyclopedia, Noyes Development Corporation, Park Ridge, N.J., 1968.

62. Garrision, W. E., J. G. Kremer, J. Murk, "Improved Hypochlorination Techniques and Problems in Disinfection of Municipal Wastewaters Containing Refinery Thiosulfate", Ind. Waste Conf., 28:309-322, 1973.

63. Sulfur Oxide Control Technology Assessment Panel (SOCTAP), Projected Utilization of Stack Gas Cleaning Systems by Steam-Electric Plants, Federal Interagency Committee for Evaluation of State Air Implementation Plans, Washington, D. C., 1973.

64. Bischoff, W. F., P. Steiner, "Coal Converts SO_2 to S", Chemical Engineering, 82(1):74-75, 1975.

65. National Air Pollution Control Administration, Nationwide Inventory of Air Pollutant Emissions 1968, Washington, D. C.

66. Manderson, M. C., "The Sulfur Outlook", in Sulfur and SO_2 Developments, AIChE, New York, 1971.

67. U. S. Environmental Protection Agency, Technology Transfer Division, Process Design Manual for Nitrogen Control, Washington, D. C., 1975.

68. U. S. Environmental Protection Agency, Technology Transfer Division, Nitrification and Denitrification Facilities Wastewater Treatment, Washington, D. C., 1973.

69. Chemical Marketing Reporter, March 22, 1976.

70. Chemical Marketing Reporter, July 7, 1975.

71. Schroeder, H. A., "Relation Between Mortality from Cardiovascular Disease and Treated Water Supplies", J. Am. Med. Assoc., 172(17):1902-1908, 1960.

72. U. S. Public Health Service, "Drinking Water Standards", Federal Register, 27(44):2152, 1962.

73. McKee, J. E., H. W. Wolf, Water Quality Criteria, 2nd Ed., The Resources Agency of California, State Water Quality Control Board, Publication No. 3-A, 1963.

74. U. S. Environmental Protection Agency, "National Interim Primary Drinking Water Regulations", Federal Register, 40(248):59566-59588, 1975.

75. Olson, D. M., The Effect of Sulfate and Manganese Dioxide on the Release of Phosphorus from Lake Mendota Sediments, Water Resources Center, Wisconsin University, Madison, Wis., abstracted in Government Reports Announcements & Index, 75(21):66, 1975.

76. Riley, J. P., G. Skirrow, Chemical Oceanography, Vol. 1, Academic Press, New York, 1965.

APPENDICES

APPENDIX A1. ELEMENTAL SULFUR ANALYSIS

A. Reagents

1. Sodium Sulfite, 100 g/ℓ
2. Formaldehyde solution, 37%; with 10-15% methanol as preservative
3. 0.25 N Iodine solution
4. 0.025 N Iodine solution
5. 1.0 N Sodium Thiosulfate
6. Acetic Acid
7. Starch indicator
8. Antifoam spray

B. Procedure

1. Pipette an aliquot from a well stirred sample into a 250 ml erlenmeyer flask using a broken tipped pipette.
2. Add 50 ml sodium sulfite solution. If more than 300 mg sulfur are in the sample, add 100 ml.
3. Add boiling chips to flask, spray with antifoam, and place flask on heating apparatus beneath a hood.
4. Boil for 15 minutes past the time when no sulfur particles are visible.
5. Cool, add 10 ml formaldehyde solution and 10 ml acetic acid. Add 20 ml of both reagents if 100 mℓ of the sodium sulfite solution was added.
6. Titrate contents of flask, or an aliquot of the contents, with 0.25 N iodine solution, until the light yellow iodine color begins to appear. Add a few drops of starch solution and continue titration until the blue color is stable for a few seconds. If less than

10 mg sulfur is expected, titrate with 0.025 N iodine solution.

7. Standardize iodine solution by titrating a known volume of a standard 1N thiosulfate solution using the same amounts of sodium sulfite, formaldehyde, and acetic acid as the samples.

8. Calculate sulfur concentration in mg/ℓ from:

$$S = \frac{(\text{ml titrated})}{(\text{ml of sample})} \text{ (normality of iodine solution) (32,000)}$$

APPENDIX A2. ATP ANALYSIS

A. Measurement Procedure

1. Add 2 ml cocktail mixture to glass scintillation vial.

   a. cocktail mixture

      10 ml 0.2 M Sodium Arsenate

      20 ml 0.16 M Magnesium Sulfate

      10 ml 0.2 M Tris Buffer (pH = 7.75)

      ∿ 5 ml FLE Solution

   b. FLE solution preparation

      1) remove vile of FLE-50 (Sigma Chemical Co.) from freezer and rinse contents with five 1 ml portions of distilled de-ionized water.

      2) place in manual homogenizer, put in ice-water bath and homogenize for five minutes.

      3) let sit in bath for 30 minutes, homogenize for 5 minutes, let sit for 2 hours.

      4) filter through 0.45 µm membrane filter.

2. Dilute extracted sample so that concentration is in range of 0.02-0.20 µg ATP/0.1 ml.

3. Add 0.1 ml of extracted sample (diluted if necessary), to vial, start stopwatch, swirl, place in scintillation counter.

4. After 15 seconds, switch scintillation counter to "repeat."

5. Use 5th count as measurement.

6. Make set of ATP standards and analyze during analysis period to detect deactivation of enzyme.

7. Make standard curve, adjusting for enzyme deactivation if necessary, and calculate ATP content in samples.

B. ATP Extraction Procedure

1. Filter sample through 0.45 µm membrane filter.

2. Place filter in test tube containing 7 ml Tris Buffer (0.02 M, pH 7.75) which has been in boiling water bath.

3. Replace in boiling water bath, mix occasionally.

4. After 10 minutes, remove, and cool rapidly.

5. Freeze, if analysis is not immediate.

C. Scintillation Counter Settings

1. Main power- high voltage (h.v.)

2. Preset time - 0.1 minute

3. Mode selector - auto

4. Preset count - 900

5. Gain - 100

6. Window - C-D; C = 5, D = 100

7. Sample changer - "STOP", then "RESET"

8. Coincidence switch - "OFF"

APPENDIX B. TRANSIENT RATE TEST DATA, RESULTS OF ZONE SETTLING TEST, RESULTS OF FLOCCULENT SETTLING TEST.

TABLE B1. TRANSIENT RATE TEST; θ_c = 10, S/X = 142, T = 21°C

Batch Test, N^{to} = 10.0 mg/ℓ NO_3^--N

T(min)	NO_3^--N(mg/ℓ)	NO_2^--N(mg/ℓ)
10	7.90	0.27
32	7.10	0.22
50	4.70	0.37
70	2.85	0.17
87	1.60	0.20
107	0.05	0.03

Batch Test, N^{to} = 20.0 mg/ℓ NO_3^--N

T(min)	NO_3^--N(mg/ℓ)	NO_2^--N(mg/ℓ)
3	9.16	0.50
17	8.65	0.37
21	6.96	0.60
45	4.95	0.29
60	4.31	0.24
75	2.44	0.23
92	0.99	0.17

TABLE B2. TRANSIENT RATE TEST; θ_c = 15, S/X = 150, T = 21°C

θ = 319 min, N^{to} = 9.9 mg/ℓ NO_3^--N

T(min)	NO_3^--N(mg/ℓ)	NO_2^--N(mg/ℓ)
13	8.6	0.03
39	7.1	0.07
60	4.7	0.09
79	3.8	0.10
97	4.3	0.12
116	3.6	0.18
146	1.1	1.51
170	0	1.34
199	0	0.10

θ = 362 min, N^{to} = 19.81 mg/ℓ NO_3^--N

T(min)	NO_3^--N(mg/ℓ)	NO_2^--N(mg/ℓ)
19	18.3	0.007
52	14.9	0.007
84	13.0	0.009
111	11.6	0.14
143	9.3	0.31
179	4.5	2.27
212	1.7	5.30
234	0	5.45
260	0	3.30
294	0	0.25

TABLE B3. TRANSIENT RATE TEST, S/X = 45

θ = 351 min, N^{to} = 9.96 mg/ℓ NO_3^--N

T(min)	NO_3^--N(mg/ℓ)	NO_2^--N(mg/ℓ)
9	5.90	5.0
39	1.30	9.10
69	0.30	8.20
101	0	6.08
130	0	3.52
160	0	0.90

θ = 357 min, N^{to} = 19.9 mg/ℓ NO_3^--N

T(min)	NO_3^--N(mg/ℓ)	NO_2^--N(mg/ℓ)
9	13.2	4.72
39	7.7	11.20
69	1.9	15.45
99	0	16.90
129	0	15.90
159	0	15.10
189	0	13.15
219	0	11.60
249	0	9.88
279	0	7.35
309	0	5.12
339	0	2.70
369	0	0.60

TABLE B4. TRANSIENT RATE TEST DATA; S/X = 56

θ = 359 min, N^{to} = 10.0 mg/ℓ NO_3^--N

T(min)	NO_3^--N(mg/ℓ)	NO_2^--N(mg/ℓ)
9	6.8	2.65
40	1.6	9.50
70	0	11.90
100	0	11.25
129	0	10.45
164	0	8.95
189	0	7.55
219	0	6.20
249	0	4.82
279	0	3.65
308	0	2.25
343	0	0.71

θ = 337 min, N^{to} = 20.0 mg/ℓ NO_3^--N

T(min)	NO_3^--N(mg/ℓ)	NO_2^--N(mg/ℓ)
7	12.5	3.80
38	6.65	11.05
68	1.69	16.65
96	0	18.60
126	0	18.00
158	0	15.75
186	0	14.35
218	0	12.25
247	0	10.95
277	0	9.20
306	0	7.92

TABLE B5. TRANSIENT RATE TEST DATA: S/X = 100

θ = 347 min, N^{to} = 10.25 mg/ℓ NO_3^--N

T(min)	NO_3^--N(mg/ℓ)	NO_2^--N(mg/ℓ)
14	2.10	6.00
44	0.50	10.55
71	0	9.75
103	0	6.95
134	0	4.05
162	0	1.10

θ = 350 min, N^{to} = 20.5 mg/ℓ NO_3^--N

T(min)	NO_3^--N(mg/ℓ)	NO_2^--N(mg/ℓ)
10	11.6	5.78
40	6.6	13.82
70	1.8	19.80
103	0	18.90
132	0	17.12
161	0	14.01
190	0	11.12
221	0	7.40
250	0	4.30
280	0	1.35

TABLE B6. TRANSIENT RATE TEST DATA: S/X = 194

θ = 332 min, N^{to} = 9.75 mg/ℓ NO_3^--N

T(min)	NO_3^--N(mg/ℓ)	NO_2^--N(mg/ℓ)
11	4.26	5.42
42	1.00	9.75
71	0	8.55
101	0	5.95
131	0	3.9
165	0	1.80
190	0	0.37

θ = 309 min, N^{to} = 18.5 mg/ℓ NO_3^--N

T(min)	NO_3^-N(mg/ℓ)	NO_2^--N(mg/ℓ)
8	12.50	5.60
39	1.13	14.05
69	0	17.75
97	0	15.95
127	0	13.28
159	0	10.15
187	0	7.80
219	0	4.90
248	0	3.62
278	0	2.14
301	0	1.12

TABLE B7. TRANSIENT RATE TEST DATA; $T = 12^{o}C$

θ = 382 min, N^{to} = 9.45 mg/ℓ NO_3^--N

T(min)	NO_3^--N(mg/ℓ)	NO_2^--N(mg/ℓ)
12	6.8	0.04
40	6.3	0.06
71	4.7	0.06
100	3.8	0.04
130	2.5	0.04
162	1.6	0.03
190	0.76	0.04

θ = 384 min, N^{to} = 18.9 mg/ℓ NO_3^--N

T(min)	NO_3^--N(mg/ℓ)	NO_2^--N(mg/ℓ)
10	13.9	0.04
40	14.3	0.05
70	12.2	0.04
100	10.1	0.04
130	8.1	0.04
160	7.1	0.04
190	5.3	0.04
220	3.8	0.04
250	2.6	0.04
280	1.3	0.03

TABLE B8. TRANSIENT RATE TEST DATA: T = 30°C

θ = 331 min, N^{to} = 20.7 mg/ℓ NO_3^--N

T(min)	NO_3^--N(mg/ℓ)	NO_2^--N(mg/ℓ)
11	12.1	7.65
41	2.7	16.5
71	0	13.5
101	0	7.9
131	0	2.15

θ = 331 min, N^{to} = 10.25 mg/ℓ NO_3^--N

T(min)	NO_3^--N(mg/ℓ)	NO_2^--N(mg/ℓ)
13	2.6	7.5
41	0.4	7.8
72	0	3.0

TABLE B9. RESULTS OF ZONE SETTLING TEST; S/X = 45

Total suspended solids (mg/ℓ)	Zone settling velocity (ft/hr)	Solids flux (lb/ft^2-day)
18,480	18.9	522
19,640	17.2	507
21,140	16.0	506
23,230	14.1	490
24,680	13.0	481
25,660	11.6	446
28,710	10.7	463
29,940	9.74	437
33,290	9.06	452
35,990	8.58	463
40,020	7.09	425
43,520	6.57	429

TABLE 10. RESULTS OF ZONE SETTLING TEST; S/X = 56

Total suspended solids (mg/ℓ)	Zone settling velocity (ft/hr)	Solids flux (lb/ft^2-day)
32,910	16.5	812
36,400	14.4	787
38,380	12.7	728
41,520	11.2	699
46,220	9.84	681
50,080	8.44	634
54,870	7.34	604
61,170	5.81	532
68,360	4.17	427
73,840	3.62	401
87,760	2.36	311

TABLE B11. RESULTS OF ZONE SETTLING TEST; S/X = 150

Total suspended solids (mg/ℓ)	Zone settling velocity (ft/hr)	Solids flux (lb/ft^2-day)
36,630	34.4	1892
46,230	27.4	1897
52,200	21.6	1691
63,290	16.9	1602
72,280	13.4	1450
81,920	11.2	1381
95,050	9.45	1347
102,210	7.74	1189
116,360	5.98	1042
118,920	5.16	919

TABLE 12. RESULTS OF FLOCCULENT SETTLING TEST; S/X = 45

Average initial solids concentration = 14,040 mg/ℓ

Time (min)	Depth (ft)	Total suspended solids (mg/ℓ)
0	1.33	12,190
0	2.33	12,790
0	3.33	13,590
0	4.33	14,920
0	5.33	16,700
3.5	1.33	140
5.5	2.25	84
7	3.13	84
9	4.0	56
13	0.88	40
14	4.76	68
15	1.63	42
18	2.49	36
20	3.36	42
25	4.22	26
30	1.07	26
35.5	1.96	30
40	2.83	22
45	3.70	24
50	0.56	16
55.5	1.43	18
60	2.31	18
62	3.18	18

TABLE 13. RESULTS OF FLOCCULENT SETTLING TEST; S/X = 56

Average initial solids concentration = 16,210 mg/ℓ

Time (min)	Depth (ft)	Total suspended solids (mg/ℓ)
0	1.20	14,370
0	2.20	15,220
0	3.20	15,640
0	4.20	16,560
0	5.20	19,260
3.5	1.20	204
4.5	2.08	160
6.0	2.94	126
7.45	3.81	104
9.5	4.63	84
13	0.54	46
14.5	1.41	50
16	2.28	42
18	3.15	46
20	4.01	44
31	0.96	24
35	1.73	28
40	2.60	22
46	3.46	32
50	0.29	22
55	1.18	24
61	2.01	26
67	2.86	18

TABLE B14. RESULTS OF FLOCCULENT SETTLING TEST; S/X = 150

Average initial solids concentration = 35,560 mg/ℓ

Time (min)	Depth (ft)	Total suspended solids (mg/ℓ)
0	1.25	35,440
0	2.25	35,190
0	3.25	30,610
0	4.25	30,950
0	5.25	32,240
4	1.04	274
4.5	1.93	222
5	2.83	302
7	3.72	198
8	4.62	238
9.5	1.40	126
10	2.30	114
10.5	3.19	100
11	4.09	104
33	0.98	28
34	1.87	22
35	2.77	30
36	3.66	38

TABLE B15. RESULTS OF FLOCCULENT SETTLING TEST; S/X = 150

Average initial solids concentration = 67,440 mg/ℓ

Time (min)	Depth (ft)	Total suspended solids (mg/ℓ)
0	1.00	54,220
0	2.00	59,710
0	3.00	66,420
0	4.00	73,640
0	5.00	83,220
6	1.00	138
9	1.80	118
12	2.60	60
15	0.60	58
17	3.45	52
18	1.33	48
21	2.21	66
24	3.08	40
28	0.96	24
32	1.82	36
36	2.64	26
40	0.46	26
44	1.46	26
48	2.31	32